

Essential
Pathology
for Dental Students

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Essential Pathology *for Dental Students*

THIRD EDITION

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&

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Essential Pathology for Dental Students

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*Dedicated to
my wife Praveen
for her encouragement and inspiration,
and to my daughters
Tanya and Sugandha
for their patience and support*

Preface

तस्मात् असक्तः सततम् कार्यम् कर्म समाचर ।
असक्तः हि आचरन् कर्म परम् आप्नोति पुरुषः ।।

Thus do your duty without attachment
Perform the work that has to be done,
Since the person attains supreme form
By doing duty in a spirit of sacrifice.

(The Bhagvatgita: Ch III, Verse 19)

Third revised and modern edition of *Essential Pathology for Dental Students* is being presented to readers to keep pace with the advances in pathology and contemporary technology.

The first edition of this book was prepared in 1995 according to the syllabus prescribed by the Dental Council of India for BDS students of second year. Their syllabus includes mainly General Pathology and Haematology; and as an author, at that time I had felt then that there was no need to over-burden them with details of Systemic Pathology which was not included in their syllabus. However, I realised on the basis of the subsequent feedback that their quest for learning more Pathology was almost as much as MBBS students. It could be owing to two reasons: *Firstly*, BDS students study in the same campuses as MBBS students in vast majority of colleges and share their study material, and are also taught by the same teachers in Pathology whose expectations from both categories are almost the same. *Secondly*, there is so wide discretion with the teacher, examiner and author in giving details of examples of diseases in General Pathology (e.g. in infections, tumours) that it would really be difficult to draw a line where General Pathology ends and Systemic Pathology begins.

This shortcoming had been adequately addressed in previous editions, and now again in the third edition by including many examples of important and common diseases from Systemic Pathology.

Some *highlights* of the Third Edition are as under:

Updated contents: The subject is updated and made clearer, yet retaining its accepted style of simple and easy understanding of the contents. In general, the revised edition has a lot of recent useful information on molecular pathology and genetic basis in pathogenesis of diseases while some topics have been totally rewritten (e.g. Amyloidosis, Carcinogenesis, Lymphomas, Diabetes mellitus etc).

Organisation of the book: The revised edition has 7 sections including a section on Selected Topics from Systemic Pathology, considered relevant to learning of general processes of diseases. In view of significance of cytology in current times, a chapter on Basic Diagnostic Cytopathology has been added in the new edition.

In full colour: The entire book is now printed in full colour to cope with modern trends and needs. All the illustrations in the revised edition have been redrawn in colour which are clearer and make a pleasurable reading.

Figures and Tables: There is addition of many new tables, figures and graphic representations to enrich the student with a lot of material in short space and for quick grasp.

Colour Atlas: The Colour Atlas given in beginning of the book has 48 representative photomicrographs of high quality presented in labelled format for easy identification of the structures by the beginners in pathology.

In spite of valuable insertions and additions, sincere effort has been made that the volume of book does not increase which has been done by selective deletion of redundant and unnecessary material. In essence, the revised edition is a complete text of pathology for students of dentistry, and it is anticipated that the users would not be required to refer to any other book during their routine learning of pathology.

ACKNOWLEDGEMENTS

In preparing the revised third edition, suggestions and corrections received from my colleagues and students have been carefully taken into consideration which is gratefully acknowledged. I acknowledge the assistance by my energetic and enthusiastic colleague, Dr Amanjit, MD, DNB, Senior Lecturer, for her selfless and untiring help in giving suggestions from time to time and for proof-reading. Liberal and skillful technical assistance rendered by Ms Agam Verma, BSc (MLT), Senior Laboratory Technician, and art work by Mr Satish Kaushik, BFA, Senior Artist, have contributed in giving a presentable appearance to the book.

Constant strategic support and encouragement received from the Department of Medical Education and Research, Chandigarh Administration, Chandigarh, from time to time in completion of this work is gratefully acknowledged.

Lastly, my special thanks to the team of dedicated staff of M/s Jaypee Brothers Medical Publishers Private Ltd., Delhi, in general, and Mrs Y Kapoor, Senior Desktop Operator, in particular for acceding to my requests for amendments and insertions till the very last minute, to Mr Tarun Duneja, General Manager (Publishing), for being so very cooperative, and to Mr PS Ghuman, Senior Production Manager, and his team mates for being so meticulous in proof-reading. Finally, this outstanding output is the result of strong, motivated and able leadership of Shri JP Vij, Chairman and Managing Director, Jaypee Brothers Medical Publishers Pvt. Ltd., Delhi. Full credit also goes to Mr Nitin Goel, Gopsons, Noida, for the fine quality of printing in the final product.

In spite of my best efforts, element of human error is still likely; the readers are welcome to point out all such mistakes and render valuable suggestions for further improvements and shall be gratefully acknowledged.

June 2005

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Recommendations of the Dental Council of India*

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Syllabus in General Pathology for BDS Students as per Recommendations of the Dental Council of India

GENERAL PATHOLOGY

Introduction to pathology as a scientific study of disease, and some techniques used in the same.

Causes of disease with special reference to our prevailing conditions.

Cellular structure and metabolism.

Disturbances in metabolism of cells.

Retrogressive changes—Degeneration, necrosis and gangrene, amyloidosis, lipidosis and disorders of pigmentation, calcification.

Inflammation—Acute and chronic inflammation. Repair with special emphasis on repair of bones, wounds and the effects of modern treatment on repair.

Hypersensitivity and allergy.

Haemorrhage, shock, dehydration, reaction of body to injury.

Circulatory disturbances and hypertension.

Pathology of bacterial infections with reference to the common diseases prevalent in our country, e.g. pyogenic infection, enteric fever, toxæmias, tuberculosis, leprosy, syphilis and some examples of epidemic infections of public health interest and hospital infections.

Common diseases of the bone.

Injuries due to chemical and physical agents including ionising radiations.

Disturbances of nutrition with special reference to Indian conditions.

Metabolic disorders, e.g. rickets, scurvy, diabetes mellitus, etc.

General biology of tumours, spread of malignant tumours.

A course of lectures, lecture demonstrations and practicals in clinical pathology comprising of anaemias and their laboratory investigations, blood disorders including leukaemias, bleeding disorders and their investigations. Laboratory investigations commonly required by dental surgeons.

Lectures 45 hours

Practicals and demonstrations 60 hours

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- CL 2. Fatty change liver
- CL 3. Amyloidosis kidney
- CL 4. Haemosiderin pigment in liver

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- CL 5. Gangrene small bowel
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- CL 8. Haemorrhagic infarct lung

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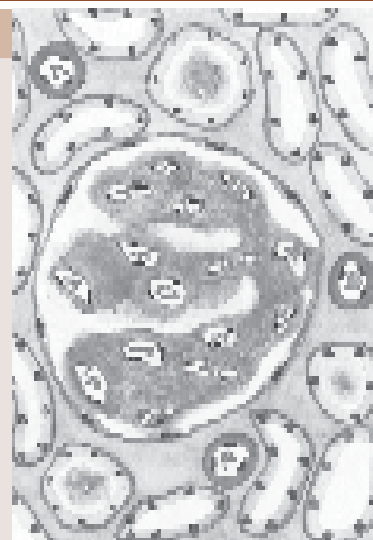
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The Cell in Health and Disease

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- CHAPTER 2: Techniques for the Study of Pathology
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- CHAPTER 6: Intracellular Accumulations
- CHAPTER 7: Amyloidosis
- CHAPTER 8: Genetic and Paediatric Diseases
- CHAPTER 9: Environmental and Nutritional Diseases



1

Introduction to Pathology

DEFINITION OF PATHOLOGY

The word '*Pathology*' is derived from two Greek words—*pathos* meaning suffering, and *logos* meaning study. Pathology is, thus, scientific study of structure and function of the body in disease; it deals with causes, effects, mechanisms and nature of disease. The knowledge and understanding of pathology is essential for all would-be doctors as well as general practitioners and specialists since unless they know the causes and mechanisms of disease and understand the language spoken by the pathologist in the form of laboratory reports, they would not be able to institute appropriate treatment or suggest preventive measures to the patient.

While medical students learn the entire subject of pathology (that includes study of General and Systemic Pathology) in their second phase, undergraduate students of dentistry are taught the subject of pathology in two phases: General Pathology in their second year and Oral Pathology in their third year; some overlapping of teaching of these subjects is therefore likely. In both

these phases, the discipline of pathology forms a vital bridge between initial learning phase of preclinical sciences and the subsequent period of clinical subjects.

HEALTH AND DISEASE

Since pathology is the study of disease, then what is *disease*? In simple language, disease is opposite of health i.e. what is not healthy is disease. *Health* is a condition when the individual is in complete accord with the surroundings, while *disease* is loss of ease to the body (dis-ease). However, it must be borne in mind that there is a wide range of 'normality' in health e.g. in height, weight, blood and tissue chemical composition etc. The confusion is further compounded by changes in health at cellular level since the cells display wide range of activities within the broad area of health similar to what is seen in diseased cells. In short, health and disease are not absolute but are considered as relative states.

A term commonly confused with disease is *illness*. While disease suggests an entity with a cause, illness is

the reaction of the individual to disease in the form of symptoms (complaints of the patient) and physical signs (elicited by the clinician).

In addition to disease and illness, there are *syndromes* (meaning running together) characterised by combination of symptoms caused by altered physiologic processes.

TERMINOLOGY IN PATHOLOGY

It is important for a beginner in pathology to be familiar with the language used in pathology:

- *Patient* is the person affected by disease.
- *Lesions* are the characteristic changes in tissues and cells produced by disease in an individual or experimental animal.
- *Pathologic changes* or *morphology* consist of examination of diseased tissues.
- The pathologic changes can be recognised with the naked eye (*gross* or *macroscopic changes*) or studied by *microscopic examination* of tissues.
- The causal factors responsible for the lesions are included in *etiology* of disease ('why' of disease).
- The mechanism by which the lesions are produced is termed *pathogenesis* of disease ('how' of disease).
- The functional implications of the lesion felt by the patient are *symptoms* and those discovered by the clinician are the *physical signs*.
- The clinical significance of the morphologic and functional changes together with results of other investigations help to arrive at an answer to what is wrong (*diagnosis*), what is going to happen (*prognosis*), what can be done about it (*treatment*), and finally what should be done to avoid complications and spread (*prevention*) ('what' of disease).

EVOLUTION OF PATHOLOGY

The concept of disease is as old as life itself. Since the beginning of mankind, there has been desire as well as need to know more about the causes and mechanisms of disease. The answers to these questions have evolved over the centuries—from supernatural beliefs to the present state of our knowledge of modern pathology.

From Religious Beliefs to Rational Approach (Antiquity to AD 1500)

The earliest concepts of disease were the religious beliefs that affliction or disease was the outcome of 'curse' or 'evil eye of spirits.' To ward them off, priests through prayers and sacrifices used to invoke supernatural powers to please the gods. The link between medicine and religion became so firmly established throughout

the world that Greeks had *Aesculapius* as the god of healing, just as orthodox Indians pray to *Mata Sheetla* as goddess of healing for cure of pox.

The period of ancient religious beliefs was followed by the philosophical and rational approach to disease by the methods of observations. This happened at the time when great Greek philosophers—*Socrates*, *Plato* and *Aristotle*, introduced philosophical concepts to all natural phenomena.

But the real practice of medicine began with *Hippocrates* (460–377 BC), the great Greek clinical genius of all times and regarded as 'the father of medicine' (Fig. 1.1). He first stressed study of patient's symptoms and described methods of diagnosis. According to him, disturbances in equilibrium of the body resulted in an illness. He recorded his observations on cases in writing which remained the mainstay of medicine for nearly two thousand years. The most famous work of Hippocrates was his aphorisms based on his vast experience, many of which have turned into medical proverbs e.g.

'Those naturally very fat are more liable to sudden death than the thin.'

'In acute diseases, coldness of extremities is bad' etc.

Hippocrates introduced ethical concepts in the practice of medicine and is revered by the medical profession by taking '*Hippocratic Oath*' at the time of entry into practice of medicine.

Hippocratic teaching was further propagated in Rome by Roman physicians, notably by *Cornelius Celsus* (53 BC–7 AD) who first described four cardinal signs of inflammation—*rubor* (redness), *tumor* (swelling), *calor* (heat), and *dolor* (pain).

The hypothesis of disequilibrium of elements constituting the body (*Dhatus*) similar to Hippocratic doctrine finds mention in ancient Indian medicine books—*Charaka Samhita*, a finest document of the rational age by *Charaka* on medicine, and *Sushruta Samhita*, similar book of surgical sciences by *Sushruta*, both of which were compiled about AD 200.

The end of Medieval period was marked by backward steps in medicine. There were widespread and devastating epidemics which reversed the process of rational thinking again to supernatural concepts and divine punishment for 'sins.'

Era of Gross Pathology (AD 1500 to 1850)

The backwardness of Medieval period was followed by the Renaissance period i.e. revival of learning. The Renaissance began from Italy in late 15th century and

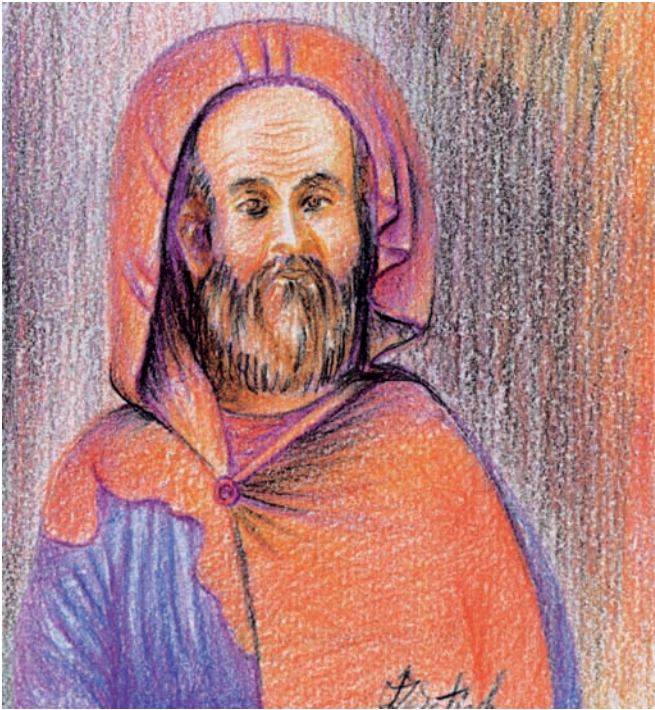


FIGURE 1.1

Hippocrates (460–377 BC). The great Greek clinical genius and regarded as ‘the father of medicine’. He introduced ethical aspects to medicine.

spread to whole of Europe. During this period, there was quest for advances in art and science. Since there was freedom of thought, there was emphasis on philosophical and rationalistic attitudes again.

The beginning of the development of human anatomy took place during this period with the art works of famous Italian painter *Leonardo da Vinci* (1452–1519). Dissection of human body was started by *Vesalius* (1514–1564) and his pupils, *Gabriel Fallopius* (1523–1562) who described human oviducts (Fallopian tubes) and *Fabricius* who discovered lymphoid tissue around the intestine of birds (bursa of Fabricius).

von Leeuwenhoek (1632–1723), draper by profession, during his spare time invented the first ever microscope by grinding the lenses himself. He also introduced histological staining in 1714 using saffron to examine muscle fibres.

The credit for beginning of the study of morbid anatomy (pathologic anatomy), however, goes to Italian anatomist-pathologist, *Giovanni B. Morgagni* (1682–1771). Morgagni was an excellent teacher in anatomy, a prolific writer as well as a practicing clinician. He published his life time experiences based on 700 postmortems and

clinical findings at the age of 79 and laid the foundations of clinicopathologic methodology in the study of disease. Thus, with Morgagni, pathology had made its beginning on the autopsy table and the concept of clinicopathologic correlation (CPC) had been introduced, establishing a coherent sequence of cause, lesions, symptoms, and outcome of disease.

Sir Percival Pott (1714–1788), famous surgeon in England, identified the first ever occupational cancer in the chimney sweeps in 1775 and discovered chimney soot as the first carcinogenic agent.

John Hunter (1728–1793) was a student of Sir Percival Pott and rose to become greatest surgeon-anatomist of all times and he, together with his elder brother *William Hunter* (1718–1788) who was a reputed anatomist-obstetrician, started the first ever museum of pathologic anatomy. John Hunter made a collection of more than 13,000 surgical specimens from his flourishing practice, arranged them into separate organ systems, made comparison of specimens from animals and plants with humans, and included many clinical pathology specimens as well, and thus developed the first museum of comparative anatomy and pathology in the world which later became the Hunterian Museum in England.

Amongst many pupils of John Hunter was *Edward Jenner* (1749–1823). In fact, Jenner’s work on inoculation in smallpox can be traced back to the earlier experiment done by John Hunter on himself by self-inoculation of a venereal lesion from a prostitute on his own glans by a lancet which resulted in delay of his marriage for three years for getting cured.

R.T.H. Laennec (1781–1826), French physician, dominated the early part of 19th century by his numerous discoveries. He described several lung diseases (tubercles, caseous lesions, miliary lesions, pleural effusion, bronchiectasis), chronic sclerotic liver disease (later called Laennec’s cirrhosis) and invented stethoscope.

Morbid anatomy attained its zenith with appearance of *Carl F. von Rokitsky* (1804–1878), self-taught German pathologist who performed nearly 30,000 autopsies himself. He described acute yellow atrophy of the liver, wrote an outstanding monograph on diseases of arteries and congenital heart defects.

The era of gross pathology had three more illustrious and brilliant physician-pathologists in England who were colleagues at Guy’s Hospital in London:

■ *Richard Bright* (1789–1858) who described non-suppurative nephritis, later termed glomerulonephritis or Bright’s disease;

■ *Thomas Addison* (1793–1860) who gave an account of chronic adrenocortical insufficiency termed Addison’s disease; and

■ *Thomas Hodgkin* (1798–1866), who observed the complex of chronic enlargement of lymph nodes, often with enlargement of the liver and spleen, later called Hodgkin's disease.

Era of Technology Development and Cellular Pathology (AD 1850 to 1950s)

Upto middle of the 19th century, correlation of clinical manifestations of disease with pathological findings at autopsy became the major method of study of disease. Sophistication in surgery led to advancement in pathology. The anatomist-surgeons of earlier centuries got replaced largely with surgeon-pathologists in the 19th century.

Pathology started developing as a diagnostic discipline in later half of the 19th century with the evolution of cellular pathology which was closely linked to technology advancements in machinery manufacture for cutting thin sections of tissue, improvement in microscope, and development of chemical industry and dyes for staining.

The discovery of existence of disease-causing micro-organisms was made by French chemist *Louis Pasteur* (1822–1895). Subsequently, *G.H.A. Hansen* (1841–1912) identified Hansen's bacillus as causative agent for leprosy (Hansen's disease) in 1873. While the study of infectious diseases was being made, the concept of immune tolerance and allergy emerged which formed the basis of immunisation initiated by Edward Jenner.

Developments in chemical industry helped in switch over from earlier dyes of plant and animal origin to synthetic dyes. This led to emergence of a viable dye industry for histological and bacteriological purposes. The impetus for the flourishing and successful dye industry came from the works of numerous pioneers as under:

■ *Paul Ehrlich* (1854–1915), German physician, conferred Nobel Prize for his work in immunology, described Ehrlich's test for urobilinogen using Ehrlich's aldehyde reagent, staining techniques of cells and bacteria, and laid the foundations of haematology;

■ *Christian Gram* (1853–1938), Danish physician, who developed bacteriologic staining by crystal violet;

■ *D.L. Romanowsky* (1861–1921), Russian physician, who developed stain for peripheral blood film using eosin and methylene blue derivatives;

■ *Robert Koch* (1843–1910), German bacteriologist who, besides Koch's postulate and Koch's phenomena, developed techniques of fixation and staining for identification of bacteria, discovered tubercle bacilli in 1882 and cholera vibrio organism in 1883;

■ *May-Grunwald* in 1902 and *Giemsa* in 1914 developed blood stains and applied them for classification of blood cells and bone marrow cells;

■ *Sir William Leishman* (1865–1926) who described Leishman's stain for blood films in 1914 and observed Leishman-Donovan bodies (LD bodies) in leishmaniasis; and

■ *Robert Feulgen* (1884–1955) who described Feulgen reaction for DNA staining and laid the foundations of cytochemistry and histochemistry.

Simultaneous technological advances in machinery manufacture led to development and upgradation of microtomes for obtaining thin sections of organs and tissues for staining by dyes for enhancing detailed study of sections.

Rudolf Virchow (1821–1905) in Germany is credited with the beginning of histopathology as a method of investigation by examination of diseased tissues at cellular level. Virchow gave two major hypotheses:

■ All cells come from other cells.

■ Disease is an alteration of normal structure and function of these cells.

Virchow is aptly known as the 'father of cellular pathology' (Fig. 1.2). Thus, sound foundation of diagnostic pathology had been laid which was followed and promoted by numerous brilliant successive workers. Virchow also described etiology of embolism (Virchow's triad—slowing of blood stream, changes in the vessel wall, changes in the blood itself), metastatic spread of tumours (Virchow's lymph node), and diseases of blood (especially leukaemias).

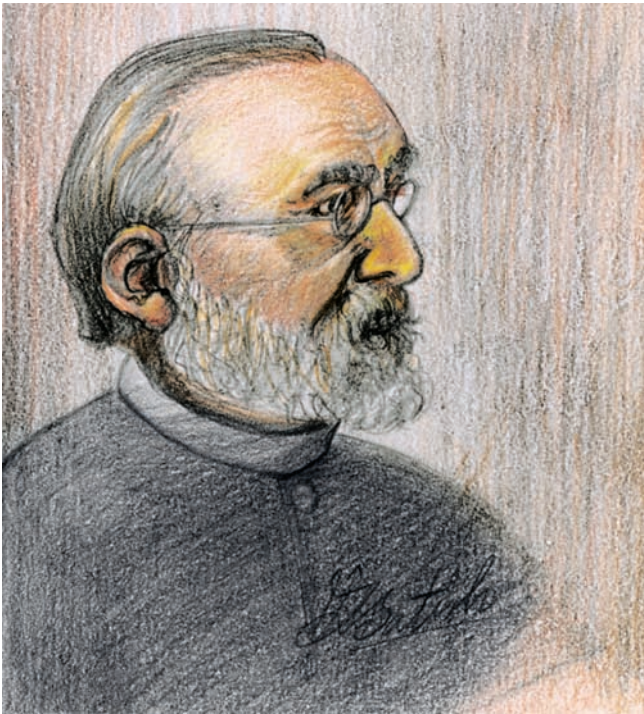
Until the end of the 19th century, the study of morbid anatomy had remained largely autopsy-based and thus had remained a retrospective science. Soon, knowledge and skill gained by giving accurate diagnosis on postmortem findings was applied to surgical biopsy and thus emerged the discipline of surgical pathology.

The concept of frozen section examination when the patient was still on the operation table had been introduced by Virchow's student, *Julius Cohnheim* (1839–1884). In fact, during the initial period of development of surgical pathology around the turn of the 19th century, frozen section was considered more acceptable by the surgeons.

Other landmarks in further evolution of modern pathology in this era are as follows:

■ *Karl Landsteiner* (1863–1943) described the existence of human blood groups in 1901 and was awarded Nobel Prize in 1930.

■ *Ruska* and *Lorries* in 1933 developed electron microscope which aided the pathologist to view ultrastructure of cell and its organelles.

**FIGURE 1.2**

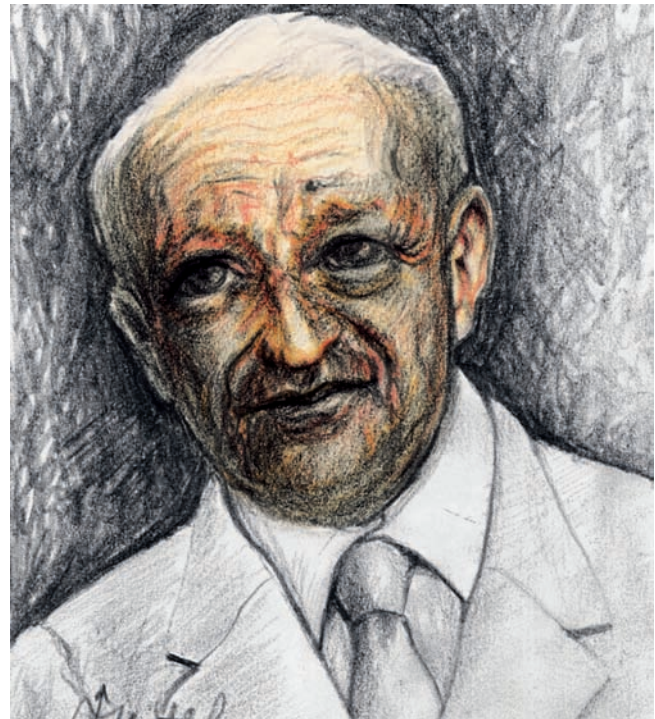
Rudolf Virchow (1821-1905). German pathologist regarded as 'the father of cellular pathology'.

■ The development of exfoliative cytology for early detection of cervical cancer began with *George N. Papanicolaou* (1883–1962), a Greek-born American pathologist, in 1930s who is known as 'father of exfoliative cytology' (Fig. 1.3).

Another pioneering teacher in pathology in the 20th century was *William Boyd* (1885–1979), psychiatrist-turned pathologist whose textbooks—'Pathology for Surgeons' (first edition 1925) and 'Textbook of Pathology' (first edition 1932), dominated and inspired the students of pathology all over the world due to his flowery language and lucid style for about 50 years till 1970s. *M.M. Wintrobe* (1901–1986), a pupil of Boyd who discovered haematocrit technique, regarded him as a very stimulating teacher with keen interest in the development of museum.

Modern Pathology (1950s to dawn of 21st century)

The strides made in the latter half of 20th century until the beginning of 21st century have made it possible to study diseases at molecular level, and provide an evidence-based and objective diagnosis and therapy. The

**FIGURE 1.3**

George N. Papanicolaou (1883-1962). American pathologist, who developed Pap test for diagnosis of cancer of uterine cervix and regarded as 'father of exfoliative cytology.'

major impact of advances in molecular biology are in the field of diagnosis and treatment of genetic disorders, immunology and in cancer. Some of the revolutionary discoveries during this time are as under:

- Description of the structure of DNA of the cell by *Watson and Crick* in 1953.
- Identification of chromosomes and their correct number in humans (46) by *Tijo and Levan* in 1956.
- Identification of Philadelphia chromosome t(9;22) in chronic myeloid leukaemia by *Nowell and Hagerford* in 1960 as the first chromosomal abnormality in any cancer.
- *In Situ Hybridization* introduced in 1969 in which a labeled probe is employed to detect and localize specific RNA or DNA sequences 'in situ' (i.e. in the original place).
- *Recombinant DNA technique* developed in 1972 using restriction enzymes to cut and paste bits of DNA.
- In 1983, *Kary Mullis* introduced *polymerase chain reaction* (PCR) i.e. "xeroxing" DNA fragments which revolutionised the diagnostic molecular genetics.
- The flexibility and dynamism of DNA invented by *Barbara McClintock* for which she was awarded Nobel Prize in 1983.

■ In 1997, *Ian Wilmut*, a Scottish scientist and his colleagues at Roslin Institute in Edinburgh, successfully used a technique of somatic cell nuclear transfer to create the clone of a sheep; the cloned sheep was named Dolly. This has set in the era of *mammalian cloning*.

■ In June 2000, discovery of chemicals of approximately 30,000 genes that make up the human body, their structure and position on chromosomes (i.e. *mapping of the human genome*) has been successfully carried out. The functions of most of the genes that comprise human genome have also been identified. All this has opened new ways in treating and researching an endless list of diseases that are currently incurable.

■ Recent report (2005) suggests that Prof Wilmut's group, which first cloned the sheep Dolly, has been granted licence from the regulatory authorities for *therapeutic cloning of human embryos* for use in treating motor neuron disease, and the embryo will be destroyed after therapeutic use. This stage of molecular biology has been reached due to availability of *human stem cell research* in which embryonic stem cells obtained from *in vitro* fertilisation will be used for cell therapy e.g. introducing insulin-producing cells into the pancreas in a patient of insulin-dependent diabetes mellitus, or using embryonic stem cells cultured in the laboratory in lieu of a whole organ transplant. It seems that time is not far when organs for transplant may be 'harvested' from the embryo.

These inventions have set in an era of human molecular biology which is no longer confined to research laboratories but is ready for application as a modern diagnostic and therapeutic tool. Modern day human molecular biology is closely linked to information technology; the best recent example is the availability of molecular profiling by *cDNA microarrays* in which by a small silicon chip, expression of thousands of genes can be simultaneously measured.

SUBDIVISIONS OF PATHOLOGY

After a retrospective into the historical aspects of pathology, and before plunging into the study of pathology in the chapters that follow, we first introduce ourselves with the branches of human pathology.

Human pathology is the largest branch of pathology. It is conventionally divided into *General Pathology* dealing with general principles of disease, and *Systemic Pathology* that includes study of diseases pertaining to the specific organs and body systems. With the advancement of diagnostic tools, the broad principles of which are outlined in the next chapter, the speciality

of pathology has come to include the following subspecialties:

A. HISTOPATHOLOGY. Histopathology, used synonymously with anatomic pathology, pathologic anatomy, or morbid anatomy, is the classic method of study and still the most useful one which has stood the test of time. The study includes structural changes observed by naked eye examination referred to as gross or macroscopic changes, and the changes detected by light and electron microscopy supported by numerous special staining methods including histochemical and immunological techniques to arrive at the most accurate diagnosis. Modern time anatomic pathology includes subspecialties such as cardiac pathology, pulmonary pathology, neuropathology, renal pathology, gynaecologic pathology, dermatopathology, gastrointestinal pathology, oral pathology, and so on. Anatomic pathology includes the following 3 main subdivisions:

1. *Surgical pathology* deals with the study of tissues removed from the living body. It forms the bulk of tissue material for the pathologist and includes study of tissue by paraffin embedding techniques and by frozen section for rapid diagnosis.

2. *Forensic pathology and autopsy work* includes the study of organs and tissues removed at postmortem. In this, the pathologist attempts to reconstruct the course of events how they happened in the patient and culminated in his death. The postmortem anatomic diagnosis is helpful to the clinician to enhance his knowledge about the disease and his judgement. The significance of a careful postmortem examination can be summed up in the old saying 'the dead teach the living'.

3. *Cytopathology*, though a branch of anatomic pathology, has developed as a distinct subspecialty in recent times. It includes study of cells shed off from the lesions (exfoliative cytology) and fine-needle aspiration cytology (FNAC) of superficial and deep-seated lesions for diagnosis (Chapter 38).

B. HAEMATOLOGY. Haematology deals with the diseases of blood. It includes laboratory haematology and clinical haematology; the latter covers the management of patient as well.

C. CHEMICAL PATHOLOGY. Analysis of biochemical constituents of blood, urine, semen, CSF etc. is included in this branch of pathology.

D. IMMUNOLOGY. Detection of abnormalities in the immune system of the body comprises immunology and immunopathology.

E. EXPERIMENTAL PATHOLOGY. This is defined as production of disease in the experimental animal and its study. However, all the findings of experimental work in animals may not be applicable to human beings due to species differences.

F. GEOGRAPHIC PATHOLOGY. The study of differences in distribution of frequency and type of diseases in populations in different parts of the world forms geographic pathology.

G. MEDICAL GENETICS. This is the branch of human genetics that deals with the relationship between

heredity and disease. There have been important developments in the field of medical genetics e.g. in blood groups, inborn errors of metabolism, chromosomal aberrations in congenital malformations and neoplasms etc.

H. MOLECULAR PATHOLOGY. The detection and diagnosis of abnormalities at the level of DNA of the cell is included in molecular pathology. Recent advancements in molecular biologic techniques have resulted in availability of these methods not only for research purposes but also as a tool in diagnostic pathology.



Techniques for the Study of Pathology

For learning contemporary pathology effectively, it is essential that the student is familiar with the various laboratory methods, techniques and tools employed for the study of pathology. This chapter is devoted to the basic aspects of various such methods as are available—ranging from the basic microscopy to the most recent methods, in a modern pathology laboratory.

AUTOPSY PATHOLOGY

Professor William Boyd in his unimitable style wrote 'Pathology had its beginning on the autopsy table'. The significance of study of autopsy in pathology is summed up in Latin inscription in an autopsy room reproduced in English as 'The place where death delights to serve the living'. As stated in the previous chapter, G.B. Morgagni in Italy (1682-1771) and T.H.A. Laennec (1781-1826) in France started collecting the case records of hospital cases and began correlation of clinical features with the lesions observed at autopsy and thus marked the beginning of clinicopathologic correlation (CPC). CPC continues to be the most important form of clinical teaching activity in medical institutions worldwide.

There is still no substitute for a careful postmortem examination which enlightens the clinician about the pathogenesis of disease, reveals hazardous effects of therapy administered, and settles the discrepancies finally between antemortem and postmortem diagnosis.

Traditionally, there are two methods for carrying out autopsy, either of which may be followed:

1. Block extraction of abdominal and thoracic organs.
2. *In situ* organ-by-organ dissection.

In conditions where multiple organs are expected to be involved, complete autopsy should be performed. But if a particular organ-specific disease is suspected, a mini-autopsy or limited autopsy may be sufficient.

The study of autopsy throws new light on the knowledge and skills of both physician as well as pathologist. The main **purposes** of autopsy are as under:

1. *Quality assurance of patientcare* by:
 - i) confirming the cause of death;
 - ii) establishing the final diagnosis; and
 - iii) study of therapeutic response to treatment.

2. *Education of the entire team* involved in patientcare by:

- i) making autopsy diagnosis of conditions which are often missed clinically e.g. pneumonia, pulmonary embolism, acute pancreatitis, carcinoma prostate;
- ii) discovery of newer diseases made at autopsy e.g. Reye's syndrome, Legionnaire's disease, SARS;
- iii) study of demography and epidemiology of diseases; and
- iv) affords education to students and staff of pathology.

SURGICAL PATHOLOGY

Surgical pathology is the classic and time-tested method of tissue diagnosis made on gross and microscopic study of tissues.

Technology development and advances made in the dye industry in the initial years of this century have made, the speciality of diagnostic surgical pathology by biopsy as ultimate diagnostic science.

SCOPE AND LIMITATIONS OF SURGICAL PATHOLOGY

Surgical pathology services in any large hospital depend largely on inputs from surgeons and physicians familiar with the scope and limitations inherent in the speciality. Thus it is vital that clinician and pathologist communicate freely—formally as well as informally, through request forms, verbally, and at different fora such as tissue committees and interdepartmental conferences.

SURGICAL PATHOLOGY PROTOCOL

1. REQUEST FORMS. The first and foremost task of the clinician requesting tissue diagnosis is to send a completed request form that accompanies the surgical pathology specimen. It must contain the entire relevant information about the case and the disease (history, physical and operative findings, results of other relevant biochemical/haematological/radiological investigations, and clinical and differential diagnosis).

2. TISSUE ACCESSION. Tissue received in the surgical pathology laboratory must have proper identifi-

cation of the specimen matching with that on the accompanied request form. For routine tissue processing by paraffin-embedding technique, the tissue must be either in appropriate fixative solution (most commonly 10% formol-saline or 10% buffered formalin) or received fresh-unfixed. For frozen section, the tissue is always transported fresh-unfixed. The frozen section is employed for rapid diagnosis and for demonstration of certain constituents which are normally lost in processing in alcohol or xylene e.g. fat, enzymes etc. Microwave fixation may also be used in the laboratory for rapid fixation of routine surgical specimens.

3. GROSS ROOM. Gross examination of the specimen received in the laboratory is the next most important step. Proper gross dissection, description and selection of tissue sample is a crucial part of the pathologic examination of tissue submitted. Complacency at this step cannot be remedied at a later stage and might require taking the tissue pieces afresh if the specimen is large enough and delay the report, or if the tissue biopsy is small the entire surgical procedure may have to be done again. In recent times, gross description can be recorded by a speech-recognition system without the aid of an assistant to do it.

Calcified tissues and bone are subjected to decalcification to remove the mineral and soften the tissue by treatment with decalcifying agents such as acids and chelating agents (most often aqueous nitric acid).

It is mandatory that all the gross-room personnel follow strict precautions in handling the tissues infected with tuberculosis, hepatitis, HIV and other viruses.

4. HISTOPATHOLOGY LABORATORY. Tissue cassettes along with unique number given in the gross room to the tissue sample is carried throughout laboratory procedures. Majority of histopathology departments use automated tissue processors (Fig. 2.1,A) having 12 separate stages completing the cycle in about 18 hours by overnight schedule as under:

- 10% formalin for fixation;
- ascending grades of alcohol (70%, 95% through 100%) for dehydration for about 5 hours in 6-7 jars;
- xylene/toluene/chloroform for clearing for 3 hours in two jars; and
- paraffin impregnation for 6 hours in two thermostat-fitted waxbaths.

Embedding of tissue is done in molten wax, blocks of which are prepared using L (Leuckhart's) mould. Now-a-days, plastic moulds in different colours for blocking are also available and the entire process can be temperature-controlled for which tissue embedding centres are available (Fig. 2.1,B). The blocks are then trimmed followed by sectioning by microtomy, most often by rotary microtome, employing fixed knife or disposable blades (Fig. 2.2).

Cryostat or frozen section eliminates all the steps of tissue processing and paraffin-embedding. Instead the



A



B

FIGURE 2.1

Automatic tissue processor (A) and tissue embedding centre (B) (both Thermo Shandon, UK). Courtesy: Towa Optics (India) Pvt. Ltd., New Delhi.

**FIGURE 2.2**

Rotary microtome (Thermo Shandon, UK). Courtesy: Towa Optics (India) Pvt. Ltd., New Delhi.

tissue is frozen to ice at about -25°C which acts as embedding medium and then sectioned (Fig. 2.3). Sections are then ready for staining. It is a quick diagnostic procedure for tissues before proceeding to a major radical surgery. This is also used for demonstration of some special substances in the cells and tissues e.g. fat, enzymes. This procedure can be carried out in operation theatre complex near the operating table.

Paraffin-embedded sections are routinely stained with haematoxylin and eosin (H & E). Frozen section is stained with rapid H & E or toluidine blue routinely. Special stains are employed for either of the two methods according to need. The sections are mounted and submitted for microscopic study.

5. SURGICAL PATHOLOGY REPORT. The final and the most important task of pathology laboratory is issuance of a prompt, accurate, brief, and prognostically significant report. The ideal report must contain five aspects:

- i) History (as available to the pathologist including patient's identity).
- ii) Precise gross description.
- iii) Brief microscopic findings.
- iv) Morphologic diagnosis which must include the organ for indexing purposes using SNOMED (Scientific Nomenclature in Medicine) codes.
- v) Additional comments in some cases.

SPECIAL STAINS

In H & E staining, haematoxylin stains nuclei and eosin is used as counterstain for cytoplasm and various

**FIGURE 2.3**

Cryostat (Model Cryotome, Thermo Shandon, UK). Courtesy: Towa Optics (India) Pvt. Ltd., New Delhi.

extracellular materials. H & E staining is routinely used to diagnose microscopically vast majority of surgical specimens. However, in certain 'special' circumstances when the pathologist wants to demonstrate certain specific substances/constituents of the cells/tissues to confirm etiologic, histogenic or pathogenetic components, special stains are employed. The staining depends upon either physical, chemical or differential solubility of the stain with the tissues. Some of the commonly used special stains in a surgical pathology laboratory are listed in Table 2.1.

ENZYME HISTOCHEMISTRY

Enzyme histochemical techniques for tissue section require special preparations of fresh tissues and can not be applied to paraffin-embedded sections or formalin-fixed tissues since enzymes are damaged rapidly. Currently, enzyme histochemistry has limited applications due to complex techniques and preparations required and have been largely superseded by immunohistochemical procedures and molecular pathology techniques.

Some of the commonly used enzyme histochemical stains are acid and alkaline phosphatases, acetyl cholinesterase (ACE), nonspecific esterases, tyrosinase-DOPA reaction, dehydrogenase, ATPase etc.

TABLE 2.1: Common Special Stains in Surgical Pathology (in Alphabetic Order of Constituents).

STAIN	COMPONENT/TISSUE	INTERPRETATION
A. AMYLOID		
1. <i>Congo red with polarising light</i>	Amyloid	Green-birefringence: amyloid
B. CARBOHYDRATES		
2. <i>Periodic acid-Schiff (PAS)</i>	Carbohydrates, (particularly glycogen), all mucins	Glycogen and other carbohydrates: magenta Nuclei: blue
3. <i>Mucicarmine/Best's carmine</i>	Acidic mucin	Mucin: red; Nuclei: blue
4. <i>Alcian blue (AB)</i>	Acidic mucin	Acid mucin: blue; Nuclei: red
C. CONNECTIVE TISSUES		
5. <i>Van Gieson's</i>	Extracellular collagen	Nuclei: blue/black; Collagen: red; Other tissues: yellow
6. <i>Masson's trichrome</i>	Extracellular collagen	Nuclei: blue/black Cytoplasm, muscle, red cells: red Collagen: blue
7. <i>Phosphotungstic acid-haematoxylin (PTAH)</i>	Muscle and glial tissue	Muscle striations, neuroglial fibres, fibrin: dark blue Nuclei: blue; Cytoplasm: pale pink filaments
8. <i>Verhoeff's elastic</i>	Elastic fibres	Elastic fibres: black; Other tissues: counter-stained
D. LIPIDS		
9. <i>Oil red O</i>	Fats (unfixed cryostat)	Mineral oils: red Unsaturated fats, phospholipids: pink
10. <i>Sudan Black B</i>	Fats (unfixed cryostat)	Unsaturated fats: blue black
E. MICRO-ORGANISMS		
11. <i>Gram's</i>	Bacteria (cocci, bacilli)	Gram-positive, keratin, fibrin: blue Gram-negative: red
12. <i>Ziehl-Neelsen's (Acid-fast)</i>	Tubercle bacilli	Tubercle bacilli, hair shaft, actinomyces: red Background: pale blue
13. <i>Fite-Wade</i>	Leprosy bacilli	Lepa bacilli: red; Background: blue
14. <i>Grocott's silver methanamine</i>	Fungi	Fungi, <i>Pneumocystis</i> : black; Red cells: yellow Background: pale green
F. PIGMENTS AND MINERALS		
15. <i>Perl's Prussian blue</i>	Haemosiderin, iron	Ferric iron: blue; Nuclei: red
16. <i>Masson-Fontana</i>	Melanin, argentaffin cells	Melanin, argentaffin, chromaffin, lipofuscin: black Nuclei: red
17. <i>von Kossa</i>	Mineralised bone	Mineralised bone: black; Osteoid: red
18. <i>Rubeanic acid</i>	Copper	Copper: greenish-black; Nuclei: pale red
G. PROTEINS AND NUCLEIC ACIDS		
19. <i>Feulgen reaction</i>	DNA	DNA: red purple; Cytoplasm: green
20. <i>Methyl green-pyronin</i>	DNA, RNA	DNA: green-blue; RNA: red

BASIC MICROSCOPY

Microscope is the basic tool of the pathologist just as is the stethoscope for the physician and scalpel for the surgeon. It is an instrument which produces greatly enlarged images of minute objects. The usual type of

microscope used in clinical laboratories is called *light microscope*.

In general, there are two types of light microscopes: **Simple microscope**. This is a simple hand magnifying lens. The magnification power of hand lens is from 2x to 200x.

Compound microscope. This has a battery of lenses which are fitted in a complex instrument. One type of lens remains near the object (objective lens) and another type of lens near the observer's eye (eye piece lens). The eye piece and objective lenses have different magnification. The compound microscope can be *monocular* having single eye piece or *binocular* which has two eye pieces (Fig. 2.4). *Multi-headed microscopes* are used as an aid to teaching and for demonstration purposes.

VARIANTS OF LIGHT MICROSCOPY. Besides the light microscopes, other modifications for special purposes in the clinical laboratories are as under:

Dark ground illumination (DGI). This method is used for examination of unstained living micro-organisms e.g. *Treponema pallidum*. The micro-organisms are illuminated by an oblique ray of light which does not pass through the micro-organism. The condenser is blackened in the centre and light passes through its periphery illuminating the living micro-organism on a glass slide.

Polarising microscope. This method is used for demonstration of birefringence e.g. amyloid, foreign body, hair etc. The light is made plane polarised. After passing through a disc, the rays of light vibrate in a single plane at right angle to each other. Two discs made up of prism are placed in the path of light, one below the object known as polariser and another placed in the body tube which is known as analyser. The lower disc is rotated to make the light plane polarised.

Fluorescence microscope and electron microscope are discussed separately below.

IMMUNOFLUORESCENCE

Immunofluorescence technique is employed to localise antigenic molecules on the cells by microscopic examination. This is done by using specific antibody against the antigenic molecule forming antigen-antibody complex at the specific antigenic site which is made visible by employing a fluorochrome which has the property to absorb radiation in the form of ultraviolet light so as to be within the visible spectrum of light in microscopic examination.

The immunofluorescent method has the following essential components:

FLUORESCENCE MICROSCOPE. Fluorescence microscopy is based on the principle that the exciting radiation from ultraviolet light of shorter wavelength (360 nm) or blue light (wavelength 400 nm) causes fluorescence of certain substances and thereafter re-emits light of a longer wavelength.



FIGURE 2.4

Binocular light microscope (Model E 400, Nikon, Japan). Courtesy: Towa Optics (India) Pvt. Ltd., New Delhi.

Source of light. Mercury vapour and xenon gas lamps are used as source of light for fluorescence microscopy.

Filters. A variety of filters are used between the source of light and objective: *first*, heat absorbing filter; *second*, red-light stop filter; and *third* exciter filter to allow the passage of light of only the desired wavelength. On passing through the specimen, light of both exciting and fluorescence wavelength collects. Exciter light is removed by another filter called *barrier filter* between the objective and the observer to protect the observer's eyes so that only fluorescent light reaches the eyes of observer.

Condenser. Dark-ground condenser is used in fluorescence microscope so that no direct light falls into the objective and instead gives dark contrast background to the fluorescence.

APPLICATIONS. Immunofluorescence methods are applied for the following purposes:

1. *Detection of autoantibodies in the serum* e.g. smooth muscle antibodies (SMA), antinuclear antibodies (ANA), antimitochondrial antibody (AMA), thyroid microsomal antibody etc.
2. *In renal diseases* for detection of deposits of immunoglobulins, complement and fibrin in various types of glomerular diseases by frozen section as discussed in Chapter 29.
3. *In skin diseases* to detect deposits of immunoglobulin by frozen section, particularly at the dermo-epidermal

junction and in upper dermis e.g. in various bullous dermatosis.

4. For study of *mononuclear cell surface markers* using monoclonal antibodies.

5. For specific diagnosis of *infective disorders* e.g. viral hepatitis.

IMMUNOHISTOCHEMISTRY

Immunohistochemistry is the application of immunologic techniques to the cellular pathology. The technique is used to detect the status and localisation of particular antigen in the cells by use of specific antibodies which then helps in determining cell lineage specifically, or is used to confirm a specific infection.

In the last decade, significant advances have been made in techniques for immunohistochemistry so much so that routinely processed paraffin-embedded tissue blocks can be used for the purpose, thus making profound impact on diagnostic surgical pathology. Earlier, diagnostic surgical pathology used to be considered a subjective science with inter-observer variation, particularly in borderline lesions and lesions of undetermined origin, but use of immunohistochemical techniques has added *objectivity, specificity and reproducibility* to the surgical pathologist's diagnosis.

Overview of Immunohistochemistry

■ *Chromogens* commonly used in immunohistochemical reaction are diaminobenzidine tetrahydrochloride (DAB) and aminoethyl carbazole (AEC), both of which produce stable dark brown reaction end-product.

■ Immunoperoxidase technique employing labelled antibody method to *formalin-fixed paraffin sections* is now widely used. Currently, the two most commonly used procedures are:

- i) *Peroxidase-antiperoxidase (PAP) method* in which PAP reagent is preformed stable immune-complex which is linked to the primary antibody by a bridging antibody.
- ii) *Avidin-biotin conjugate (ABC) immunoenzymatic technique* in which biotinylated secondary antibody serves to link the primary antibody to a large preformed complex of avidin, biotin and peroxidase.

■ The selection of antibody/antibodies for performing immunohistochemical staining is done after making differential diagnosis on H & E sections. Generally, a *panel of antibodies* is preferable over a single test to avoid errors.

■ Antibodies for immunohistochemistry are produced by polyclonal and *monoclonal (hybridoma) techniques*; the

latter is largely used to produce specific high-affinity antibodies. At present, vast number of antibodies against cell antigens for immunohistochemical stains are available and the list is increasing at a steady rate.

■ Immunohistochemical stains should always be done with appropriate *positive controls* i.e. tissue which is known to express particular antigen acts as a control. 'Sausage' tissue block technique combines the staining of multiple tissues in a single slide with a single staining procedure and is quite economical.

■ For interpretation of results of immunohistochemical stains, it is important to remember that *different antigens are localised at different sites in cells* and accordingly positive staining is seen and interpreted at those sites e.g. membranous staining for leucocyte common antigen (LCA); nuclear staining for progesterone receptors etc.

■ Immunohistochemical stains *cannot be applied* to distinguish between neoplastic and non-neoplastic lesions, or between benign and malignant tumours. These distinctions have to be done by traditional methods in surgical pathology.

Applications of Immunohistochemistry

At present, immunohistochemical stains are used for the following purposes, in order of utility:

1. Tumours of uncertain histogenesis. Immunohistochemical methods have brought about a revolution in approach to diagnosis of tumours of uncertain origin, primary as well as metastatic from an unknown primary tumour. A panel of antibodies is chosen to resolve such diagnostic problem cases; the selection of antibodies being made is based on clinical history, morphologic features, and results of other relevant investigations. Immunohistochemical stains for *intermediate filaments* (keratin, vimentin, desmin, neurofilaments and glial fibrillary acidic proteins) expressed by the tumour cells are of immense value besides others listed in Table 2.2.

2. Prognostic markers in cancer. The second important application of immunohistochemical stains is to predict the prognosis of tumours by identification of enzymes, tumour-specific antigens, oncogenes, tumour suppressor genes and tumour cell proliferation markers. Analysis of tumours by these methods is a significant improvement over the conventional prognostic considerations by clinical staging and histologic grading.

3. Prediction of response to therapy. Immunohistochemical methods are widely used to predict therapeutic response in two important tumours—carcinoma of breast and prostate. Both these tumours are under the

TABLE 2.2: Common Immunohistochemical Stains for Tumours of Uncertain Origin.

TUMOUR	IMMUNOSTAIN
1. <i>Epithelial tumours (Carcinomas)</i>	i) Pankeratin ii) Epithelial membrane antigen (EMA) iii) Carcinoembryonic antigen (CEA) iv) Neuron-specific enolase (NSE)
2. <i>Mesenchymal tumours (Sarcomas)</i>	i) Vimentin (general mesenchymal) ii) Desmin (for general myogenic) iii) Muscle specific actin (for general myogenic) iv) Myoglobin (for skeletal myogenic) v) Factor VIII (for vascular tumours)
3. <i>Special groups</i>	
a) Melanoma	i) Vimentin ii) S-100 iii) HMB-45 (most specific)
b) Lymphoma	i) Leucocyte common antigen (LCA/CD45) ii) Pan-B iii) Pan-T iv) RS cell marker (for Hodgkin's)
c) Neural and neuroendocrine tumours	i) Neurofilaments (NF) ii) NSE iii) GFAP (for glial tumours) iv) Chromogranin (for neuroendocrine) v) Synaptophysin

growth regulation of hormones—oestrogen and androgen, respectively. The specific receptors for these growth regulating hormones are located on respective tumour cells. Tumours expressing high level of receptor positivity would respond favourably to removal of the endogenous source of such hormones (oophorectomy in oestrogen-positive breast cancer and orchiectomy in androgen-positive prostatic carcinoma), or hormonal therapy is administered to lower their levels: oestrogen therapy in prostatic cancer and androgen therapy in breast cancer. The results of oestrogen-receptors and progesterone-receptors in breast cancer have significant prognostic correlation, though the results of androgen-receptor studies in prostatic cancer have limited prognostic value.

4. Infections. Immunohistochemical methods are also being applied to confirm infectious agent in tissues by use of specific antibodies against microbial DNA or RNA e.g. in CMV, HPV, *Pneumocystis carinii* etc.

ELECTRON MICROSCOPY

Electron microscope (EM) first developed in 1930s in Germany has undergone modifications so as to add

extensive new knowledge to our understanding the structure and function of normal and diseased cells at the level of cell organelles. However, more recently, widespread use of diagnostic immunohistochemistry in surgical pathology has restricted the application of EM to the following areas of diagnostic pathology:

1. In renal pathology in conjunction with light microscopy and immunofluorescence.
2. Ultrastructure of tumours of uncertain histogenesis.
3. Subcellular study of macrophages in storage diseases.
4. For research purposes.

TYPES OF EM

There are two main types of EM:

1. **Transmission electron microscope (TEM).** TEM is the tool of choice for pathologist for study of ultrastructure of cell at organelle level. In TEM, a beam of electrons passes through ultrathin section of tissue. The magnification obtained by TEM is 2,000 to 10,000 times.
2. **Scanning electron microscope (SEM).** SEM scans the cell surface architecture and provides three-dimensional image. For example, for viewing the podocytes in renal glomerulus.

Technical Aspects

Following are some of the relevant salient technical considerations pertaining to EM:

1. **Fixation.** Whenever it is planned to undertake EM examination of tissue, small thin piece of tissue not more than 1 mm thick should be fixed in 2-4% buffered glutaraldehyde or mixture of formalin and glutaraldehyde. Following fixation, the tissue is post-fixed in buffered solution of osmium tetroxide to enhance the contrast.
2. **Embedding.** Tissue is plastic-embedded with resin on grid.
3. **Semithin sections.** First, semithin sections are cut at a thickness of 1 μ m and stained with methylene blue or toluidine blue. Sometimes, paraffin blocks can also be cut for EM study but generally are not quite satisfactory due to numerous artefacts. Semithin sections guide in making the differential diagnosis and in selecting the area to be viewed in ultrathin sections.
4. **Ultrathin sections.** For ultrastructural examination, ultrathin sections are cut by use of diamond knife. In order to increase electron density, thin sections may be stained by immersing the grid in solution of lead citrate and uranyl acetate.

CYTOGENETICS

Human somatic cells are diploid and contain 46 chromosomes: 22 pairs of autosomes and one pair of sex chromosomes (XX in the case of female and XY in the males). Gametes (sperm and ova) contain 23 chromosomes and are called *haploid cells*. All ova contain 23X while sperms contain 23X or 23Y chromosomes. Thus the sex of the offspring is determined by paternal chromosomal contribution i.e. if the ovum is fertilised by X-bearing sperm, female zygote results, while an ovum fertilised by Y-bearing sperm forms male zygote.

Karyotyping

Karyotype is defined as the sequence of chromosomal alignment on the basis of size, centromeric location and banding pattern. Determination of karyotype of an individual is an important tool in cytogenetic analysis. Broad outlines of karyotyping are as under:

1. Cell selection. Cells capable of growth and division are selected for cytogenetic analysis. These include: cells from amniotic fluid, chorionic villus sampling (CVS), peripheral blood lymphocytes, bone marrow, lymph node, solid tumours etc.

2. Cell culture. The sample so obtained is cultured in mitogen media. A *mitogen* is a substance which induces mitosis in the cells e.g. PPD, phytohaemagglutinin (PHA), pokeweed mitogen (PWM), tetradecanoyl-phorbol-acetate (TPA) etc. The dividing cells are then arrested in metaphase by the addition of colchicine or colcemid, both of which are inhibitory to microtubule formation. Subsequently, the cells are lysed by adding hypotonic solution.

The metaphase cells are then fixed in methanol-glacial acetic acid mixture.

3. Staining/banding. When stained, the chromosomes have the property of forming alternating dark and light bands. For this purpose, the fixed metaphase preparation is stained by one of the following banding techniques:

- Giemsa banding* or *G-banding*, the most commonly used.
- Quinacrine banding* or *Q-banding* used to demonstrate bands along chromosomes.
- Constitutive banding* or *C-banding* is used to demonstrate constitutive heterochromatin.
- Reverse staining Giemsa banding* or *R-banding* gives pattern opposite to those obtained by G-banding.

4. Microscopic analysis. Chromosomes are then photographed by examining the preparation under the

microscope. From the photograph, chromosomes are cut and then arranged according to their size, centromeric location and banding patterns. The pairs of chromosomes are identified by the arm length of chromosomes. The centromere divides the chromosome into a short upper arm called *p arm* (p for *petit* in French meaning 'short') and a long lower arm called *q arm* (letter q next to p).

Applications

The field of cytogenetics has widespread applications in diagnostic pathology (Chapter 8). In brief, karyotyping is employed for the following purposes:

i) *Chromosomal numerical abnormalities* e.g. Down's syndrome (trisomy 21 involving autosome 21), Klinefelter's syndrome (trisomy 46), Turner's syndrome (monosomy 45, XO), spontaneous abortions.

ii) *Chromosome structural abnormalities* include translocations {e.g. Philadelphia chromosome t (9;22), cri-du-chat (5p) syndrome, repeated spontaneous miscarriages}, deletions, insertions, isochromosome, and ring chromosome formation.

iii) *Cancer* is characterised by multiple and complex chromosomal abnormalities which include increase or decrease in the number as well as structural alterations in a way that chromosomal material is deleted, duplicated or rearranged.

MOLECULAR PATHOLOGY

During the last quarter of the century, rapid strides have been made in the field of molecular biology. As a result, molecular techniques which were earlier employed for research purposes only have now been made available for diagnostic purposes. These techniques detect abnormalities at the level of DNA or RNA of the cell.

Broadly speaking, all the DNA/RNA-based molecular techniques employ hybridisation technique based on recombinant technology. Specific region of DNA or RNA is detected by labelling it with a probe (*Probe* is a chain of nucleotides consisting of certain number of known base pairs). Probes are of different sizes and sources as under:

- Genomic probe* is derived from a region of DNA of cells.
- cDNA probe* is derived from RNA by reverse transcription.
- Oligonucleotide probe* is a synthetic probe contrary to genomic DNA and cDNA probe both of which are derived from cellular material.
- Riboprobe* is prepared by *in vitro* transcription system.

Molecular Methods

Following is a brief account of various molecular techniques available as diagnostic tool in surgical pathology:

1. IN SITU HYBRIDISATION. *In situ* hybridisation (ISH) is a molecular hybridisation technique which allows localisation of nucleic acid sequence directly in the intact cell (i.e. *in situ*) without DNA extraction unlike other hybridisation-based methods described below. ISH involves specific hybridisation of a single strand of a labelled nucleic acid probe to a single strand of complementary target DNA or RNA in the tissue. The end-product of hybridisation is visualised by radio-active-labelled probe (e.g. ^{32}P , ^{125}I), or non-radioactive-labelled probe (e.g. biotin, digoxigenin).

Applications. ISH is used for the following:

- i) In *viral infections* e.g. HPV, EBV, HIV, CMV, HCV etc.
- ii) In *human tumours* for detection of gene expression and oncogenes.
- iii) In *chromosomal disorders*, particularly by use of fluorescent *in situ* hybridisation (FISH).

2. FILTER HYBRIDISATION. In this method, target DNA or RNA is extracted from the tissue, which may either be fresh, frozen and unfixed tissue, or formalin-fixed paraffin-embedded tissue. Extracted target DNA or RNA is then immobilised on nitrocellulose filter or nylon. Hybridisation of the target DNA is then done with labelled probe. DNA analysis by filter hybridisation includes various methods as under:

- i) *Slot and dot blots* in which the DNA sample is directly bound to the filter without fractionation of nucleic acid size.
- ii) *Southern blot* which is similar to dot-blot but differs in performing prior DNA-size fractionation by gel electrophoresis (E.M. Southern is the name of scientist who described Southern blot technique).
- iii) *Northern blot* is similar to Southern blot but involves size fractionation of RNA (Northern is, however, opposite direction of southern and not someone's name).
- iv) *Western blot* is analogous to the previous two methods but is employed for protein fractionation and in this method antibodies are used as probes.

Applications. In view of high degree of specificity and sensitivity of the molecular hybridisation techniques, these techniques have widespread applications in diagnostic pathology:

- i) In *neoplasia*, haematologic as well as non-haematologic.

- ii) In *infectious diseases* for actual diagnosis of causative agent, epidemiologic studies and identification of newer infectious agents.

- iii) In *inherited genetic diseases* for carrier testing, prenatal diagnosis and direct diagnosis of the genetic disease.

- iv) In *identity determination* for tissue transplantation, forensic pathology, and parentage testing.

3. POLYMERASE CHAIN REACTION. Polymerase chain reaction (PCR) is a revolutionary technique for molecular genetic purpose with widespread applications in diagnostics and research. The technique is based on the *principle* that a single strand of DNA has limitless capacity to duplicate itself to form millions of copies. In PCR, a single strand of DNA generates another by DNA polymerase using a short complementary DNA fragment; this is done using a *primer* which acts as an initiating template.

A cycle of PCR consists of three steps:

- i) *Heat denaturation* of DNA (at 94°C for 60-90 seconds).
- ii) *Annealing* of the primers to their complementary sequences (at 55°C for 30-120 seconds).
- iii) *Extension* of the annealed primers with DNA polymerase (at 72°C for 60-180 seconds).

Repeated cycling can be done in automated thermal cyclers and yields large accumulation of the target sequence since each newly generated product, in turn, acts as template in the next cycle.

Applications. PCR analysis has the same applications as for filter hybridisation techniques and has many advantages over them in being more rapid, can be automated by thermal cyclers and a need for low level of starting DNA/RNA. However, PCR suffers from the risk of contamination; thus extreme caution is required in the laboratory during PCR technique.

FLOW CYTOMETRY

Flow cytometry is a more recent tool in the study of properties of cells suspended in a single moving stream. Flow cytometry, thus, overcomes the problem of subjectivity involved in microscopic examination of cells and tissues in histopathology and cytopathology.

Flow cytometer has a laser-light source for fluorescence, cell transportation system in a single stream, monochromatic filters, lenses, mirrors and a computer for data analysis. Flow cytometer acts like a cell sorter to physically sort out cells from liquid suspension flowing in a single-file. Since single-cell suspensions are required for flow cytometry, its applications are limited to flow assays e.g. leucocytes, erythrocytes and

their precursors; body fluids, and sometimes solid tissues made into cell suspensions.

Applications. Currently, flow cytometric analysis finds uses in clinical practice in the following ways:

1. *Immunophenotyping* by detailed antigenic analysis of various haematopoietic neoplasias e.g. acute and chronic leukaemias, lymphomas (Hodgkin's and non-Hodgkin's), and plasmacytic neoplasms.
2. *Diagnosis and prognostication of immunodeficiency* e.g. in AIDS by CD4 + T lymphocyte counts. Patients with CD4 + T cell counts below 500/ μ l require antiviral treatment.
3. To *diagnose the cause of allograft rejection* in renal transplantation in end-stage renal disease by CD3 + T cell counts. Patients with CD3 + T cells below 100-200/ μ l have lower risk of graft rejection.
4. *Diagnosis of autoantibodies* in ITP, and autoimmune neutropenia.
5. *Measurement of nucleic acid content* e.g. counting reticulocytes which contain RNA.
6. *DNA ploidy* studies in various types of cancers.

COMPUTERS IN PATHOLOGY LABORATORY

A busy pathology laboratory has a lot of data to be communicated to the clinicians. Pathologist too requires access to patient's data prior to reporting of results on specimens received. It is, therefore, imperative that a modern pathology laboratory has laboratory information system (LIS) which should be ideally connected to hospital information system (HIS).

Besides, the laboratory staff and doctors should have adequate computer literacy on these systems.

LIS includes: computer system, speech recognition system and image analysis system.

COMPUTER SYSTEMS. There are two main purposes of having computers in the laboratory:

- for the *billing* of patients' investigations; and
- for *reporting* of results of tests in numeric, narrative and graphic format.

Applications. Application of computers in the pathology laboratory has several advantages as under:

1. The laboratory as well as the hospital staff have access to information pertaining to the patient which helps in *improving patientcare*.
2. The *turn-around time* (i.e. time between specimen collection and reporting of results) of any test is shortened.
3. It *improves productivity* of laboratory staff at all levels who can be utilised for other jobs.
4. *Coding and indexing* of results and data of different tests is possible on computer system.
5. For *research purposes* and getting accreditation so as to get grants for research, computerised data of results are mandatory.

SPEECH RECOGNITION SYSTEM. Computer systems are now available which can recognise and transform spoken words of gross and microscopic description of reports through dictaphone into text without the use of secretarial staff.

IMAGE ANALYSERS. Image analyser is a form of computer that can capture image formed by microscopic viewing of slide and transforms it into digital image which can be used for various purposes e.g.:

1. *Morphometric studies* of cells as regards their features.
2. *Quantitative nuclear DNA ploidy* measurement.
3. *Evaluation of immunohistochemical staining quantitatively*.
4. *Storage and retrieval of laboratory data* to save time and space occupied by the records.

The concept of '*tele-pathology*' (similar to '*tele-radiology*' for filmless radiology departments) instantaneously moves information on pathology work and also pass relevant literature on-line from one place to another far off place through internet information superhighway.

cDNA MICROARRAYS ANALYSIS. This is the latest application of silicon chip technology for simultaneous analysis of large volume of data pertaining to human genes for molecular profiling of tumours. The method eliminates use of DNA probes and instead fluorescent labelling of cDNA is used. High resolution laser scanners are used for detecting fluorescent signals while level of gene expression by different samples is measured by application of bioinformatics.



Normal Cell Structure and Function

Human body, unlike unicellular amoeba, is quite complex and is made of 70,000 billion cells which comprise different tissues and organs, each of which is assigned predetermined specific function. In health, these cells remain in accord with each other.

In order to learn the fundamentals of disease processes in pathology, therefore, it is essential to have an understanding of the causes and mechanisms of cell injury and cellular adaptations. But before that, a brief review of structure of a normal mammalian cell and its functions is outlined below.

Different types of cells of the body possess features which distinguish one type from another. However, most mammalian cells have an overall common structure and function.

CELL STRUCTURE AND FUNCTION

Under normal conditions, cells are dynamic structures existing in fluid environment. A cell is enclosed by cell membrane that extends internally to enclose nucleus and various subcellular organelles suspended in cytosol (Fig. 3.1).

CELL MEMBRANE

Electron microscopy has shown that cell membrane or plasma membrane has a trilaminar structure having a total thickness of about 7.5 nm and is known as *unit membrane*. The three layers consist of two electron-dense layers separated by an electronlucent layer. Biochemically, the cell membrane is composed of complex mixture of phospholipids, glycolipids, cholesterol, proteins and carbohydrates. These layers are in a gel-like arrangement and are in a constant state of flux. The outer surface of some types of cells shows a coat of mucopolysaccharide forming a fuzzy layer called *glycocalyx*. Proteins and glycoproteins of the cell membrane may act as antigens (e.g. blood group antigens), or may form receptors (e.g. for viruses, bacterial products, hormones, immunoglobulins and many enzymes). The cell receptors are probably related to the microtubules and microfilaments of the underlying cytoplasm. The microtubules connect one receptor with the next. The microfilaments are

contractile structures so that the receptor may move within the cell membrane. Bundle of microfilaments along with cytoplasm and protein of cell membrane may form projections on the surface of the cell called *microvilli*. Microvilli are especially numerous on the surface of absorptive and secretory cells (e.g. small intestinal mucosa) increasing their surface area.

In brief, the cell membrane performs the following important functions:

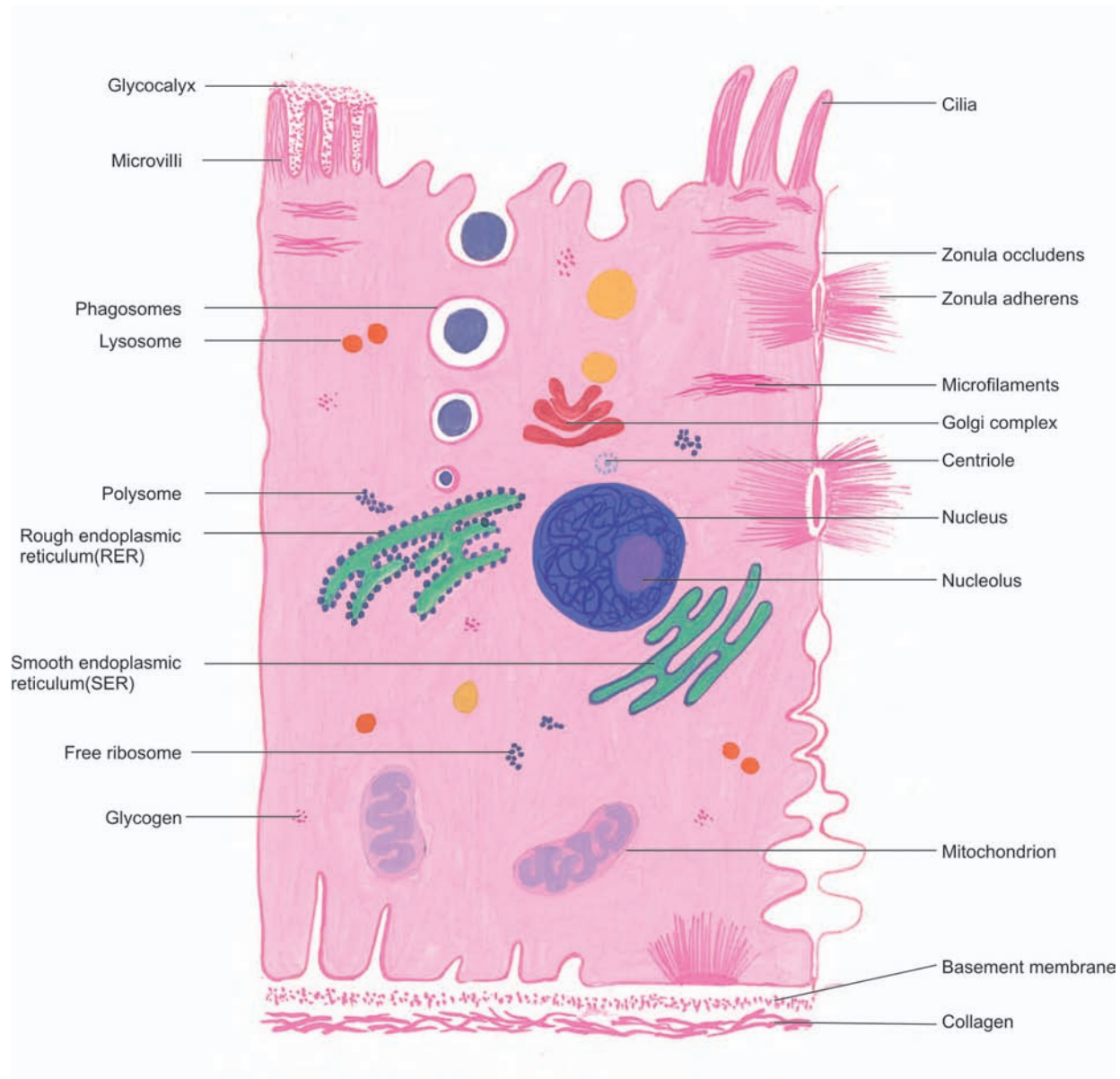
- i) Selective permeability that includes diffusion, membrane pump ('sodium pump') and pinocytosis ('cell drinking').
- ii) Membrane antigens (e.g. blood group antigens, transplantation antigen).
- iii) Cell receptors for cell-cell recognition and communication.

NUCLEUS

The nucleus consists of an outer nuclear membrane enclosing nuclear chromatin and nucleoli.

1. NUCLEAR MEMBRANE. The nuclear membrane is the outer envelop consisting of 2 layers of the unit membrane which are separated by a 40-70 nm wide space. The outer layer of the nuclear membrane is studded with ribosomes and is continuous with endoplasmic reticulum. The two layers of nuclear membrane at places are fused together forming circular nuclear pores which are about 50 nm in diameter.

2. NUCLEAR CHROMATIN. The main substance of the nucleus is comprised by the nuclear chromatin which is in the form of shorter pieces of thread-like structures called *chromosomes* of which there are 23 pairs (46 chromosomes) together measuring about a metre in length in a human diploid cell. Of these, there are 22 pairs (44 chromosomes) of *autosomes* and one pair of *sex chromosomes*, either XX (female) or XY (male). Each chromosome is composed of two chromatids connected at the centromere to form 'X' configuration having variation in location of the centromere. Depending upon the length of chromosomes and centromeric location, 46 chromosomes are categorised into 7 groups from A to

**FIGURE 3.1**

Schematic diagram of the structure of an epithelial cell.

G according to *Denver classification* (adopted at a meeting in Denver, USA).

Chromosomes are composed of 3 components, each with distinctive function. These are: deoxyribonucleic acid (DNA) comprising about 20%, ribonucleic acid (RNA) about 10%, and the remaining 70% consists of nuclear proteins that include a number of basic proteins (histones), neutral proteins, and acid proteins. DNA of the cell is largely contained in the nucleus. The only other place in the cell that contains small amount of

DNA is mitochondria. Nuclear DNA along with histone nuclear proteins form bead-like structures called nucleosomes which are studded along the coils of DNA. Nuclear DNA carries the genetic information that is passed via RNA into the cytoplasm for manufacture of proteins of similar composition. During cell division, one half of DNA molecule acts as a template for the manufacture of the other half by the enzyme, DNA polymerase, so that the genetic characteristics are transmitted to the next progeny of cells (*replication*).

The **DNA molecule** as proposed by Watson and Crick in 1953 consists of two complementary polypeptide chains forming a double helical strand which is wound spirally around an axis composed of pentose sugar-phosphoric acid chains. The molecule is spirally twisted in a ladder-like pattern the steps of which are composed of 4 *nucleotide bases*: two *purines* (adenine and guanine i.e. A and G) and two *pyrimidines* (cytosine and thymine i.e. C and T); however, A pairs specifically with T while G pairs with C (Fig. 3.2). The sequence of these nucleotide base pairs in the chain, of which there are thousands, determines the information contained in the DNA molecule or constitutes the genetic code.

In the interphase nucleus (i.e. between mitosis), part of the chromatin that remains relatively inert metabolically and appears deeply basophilic due to condensation of chromosomes is called *heterochromatin*, while the part of chromatin that is lightly stained or vesicular due to dispersed chromatin is called *euchromatin*. For example, in lymphocytes there is predominance of heterochromatin while the nucleus of a hepatocyte is mostly euchromatin.

3. NUCLEOLUS. The nucleus may contain one or more rounded bodies called nucleoli. Nucleolus is the site of synthesis of ribosomal RNA. Nucleolus is composed of granules and fibrils representing newly synthesised ribosomal RNA.

CYTOSOL AND ORGANELLES

The cytosol or the cytoplasm is the gel-like ground substance in which the organelles (meaning *little organs*) of the cells are suspended. These organelles are the site of major enzymatic activities of the cell which are possibly mediated by enzymes in the cytosol. The major organelles are the cytoskeleton, mitochondria, ribosomes, endoplasmic reticulum, Golgi apparatus or complex, lysosomes, and microbodies or peroxisomes.

1. CYTOSKELETON. Microfilaments, intermediate filaments, and microtubules are responsible for maintaining cellular form and movement and are collectively referred to as cytoskeleton.

i) Microfilaments are long filamentous structures having a diameter of 6-8 nm. They are composed of contractile proteins, actin and myosin, and diverse materials like parts of microtubules and ribonucleo-protein fibres. Bundles of microfilaments are especially prominent close to the plasma membrane and form *terminal web*. Extension of these bundles of microfilaments along with part of plasma membrane on the surface of the cell form *microvilli* which increase the absorptive surface of the cells.

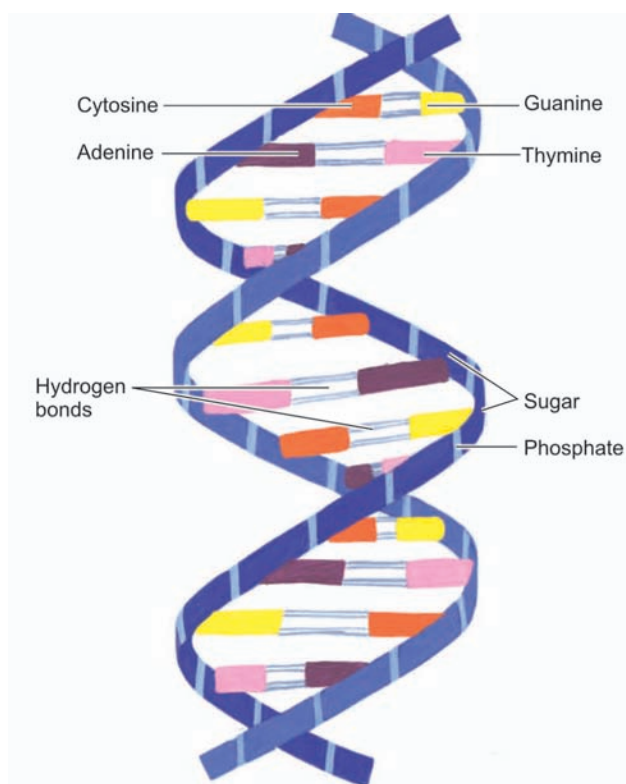


FIGURE 3.2

Diagrammatic structure of portion of DNA molecule.

ii) Intermediate filaments are filamentous structures, 10 nm in diameter, and are cytoplasmic constituent of a number of cell types. They are composed of proteins. There are 5 principal types of intermediate filaments:

- Cytokeratin* (found in epithelial cells).
- Desmin* (found in skeletal, smooth and cardiac muscle).
- Vimentin* (found in cells of mesenchymal origin).
- Glial fibrillary acidic protein* (present in astrocytes and ependymal cells).
- Neurofilaments* (seen in neurons of central and peripheral nervous system).

Their main function is to mechanically integrate the cell organelles within the cytoplasm.

iii) Microtubules are long hollow tubular structures about 25 nm in diameter. They are composed of protein, tubulin. *Cilia* and *flagella* which project from the surface of cell are composed of microtubules enclosed by plasma membrane and are active in locomotion of the cells. *Basal bodies* present at the base of each cilium or flagellum and *centriole* located at the mitotic spindle of cells are the two other morphologically similar structures composed of microtubules.

2. MITOCHONDRIA. Mitochondria are oval structures and are more numerous in metabolically active cells. They are enveloped by two layers of membrane—the outer smooth and the inner folded into incomplete septa or sheaf-like ridges called *cristae*. Chemically and structurally, membranes of mitochondria are similar to cell membrane. The inner membrane, in addition, contains lollipop-shaped globular structures projecting into the matrix present between the layers of membrane. The matrix of the mitochondria contains enzymes required in the Krebs' cycle by which the products of carbohydrate, fat and protein metabolism are oxidised to produce energy which is stored in the form of ATP in the lollipop-like globular structures. Mitochondria are not static structures but undergo changes in their configuration during energised state by alteration in matrix and intercrystal space; the outer membrane is, however, less elastic. Aside from their role in metabolism, mitochondria possess DNA and ribosomes but no histones, and may have a role in synthesising membrane-bound proteins of mitochondria.

3. RIBOSOMES. Ribosomes are spherical particles which contain 80-85% of the cell's RNA. They may be present in the cytosol as 'free' unattached form, or in 'bound' form when they are attached to membrane of endoplasmic reticulum. They may lie as 'monomeric units' or as 'polyribosomes' when many monomeric ribosomes are attached to a linear molecule of messenger RNA. Ribosomes are the protein-synthesising units.

4. ENDOPLASMIC RETICULUM. Endoplasmic reticulum is composed of vesicles and intercommunicating canals whose main function is the manufacture of protein. It is composed of unit membrane which is continuous with both nuclear membrane and the Golgi apparatus, and possibly with the cell membrane. Morphologically, there are 2 forms of endoplasmic reticulum: rough or granular, and smooth or agranular.

i) *Rough endoplasmic reticulum (RER)* is so-called because its outer surface is rough or granular due to attached ribosomes on it. RER is especially well developed in cells active in protein synthesis e.g. Russell bodies of plasma cells, Nissl granules of nerve cells.

ii) *Smooth endoplasmic reticulum (SER)* is devoid of ribosomes on its surface.

SER and RER are generally continuous with each other. SER contains many enzymes which metabolise drugs, steroids, cholesterol, and carbohydrates and partake in muscle contraction.

5. GOLGI APPARATUS OR COMPLEX. The Golgi apparatus or complex is generally located close to the

nucleus. Morphologically, it appears as vesicles, sacs or lamellae composed of unit membrane and is continuous with the endoplasmic reticulum. The Golgi apparatus is particularly well developed in exocrine glandular cells. Its main functions are synthesis of carbohydrates and complex proteins and packaging of proteins synthesised in the RER into vesicles. Some of these vesicles may contain lysosomal enzymes and specific granules such as in neutrophils and in beta cells of the pancreatic islets.

6. LYSOSOMES. Lysosomes are rounded to oval membrane-bound organelles containing powerful lysosomal digestive (hydrolytic) enzymes. There are 3 forms of lysosomes:

i) *Primary lysosomes or storage vacuoles* are formed from the various hydrolytic enzymes synthesised by the RER and packaged in the Golgi apparatus.

ii) *Secondary lysosomes or autophagic vacuoles* are formed by fusion of primary lysosomes with the parts of damaged or worn-out cell components.

iii) *Residual bodies* are indigestible materials in the lysosomes e.g. lipofuscin.

7. CENTRIOLE OR CENTROSOME. These are seen as two small structures composed of electron-dense fibrils. They perform the function of formation of cilia and flagellae and form the spindle of fibrillary protein during mitosis.

INTERCELLULAR JUNCTIONS

Plasma membranes of epithelial and endothelial cells, though closely apposed, are separated from each other by 20 nm wide space. These cells communicate across this space through intercellular junctions or junctional complexes visible under electron microscope and are of 4 types (Fig. 3.3):

1. Occluding junctions (Zonula occludens). These are tight junctions situated just below the luminal margin of adjacent cells. As a result, the regions of occluding zones are impermeable to macromolecules. The examples of occluding zones are seen in renal tubular epithelial cells, intestinal epithelium, and vascular endothelium in the brain constituting blood-brain barrier.

2. Adhering junctions (Zonula adherens). These are located just below the occluding zones between the adjacent cells and are permeable to tracer particles. These zones are in contact with actin microfilaments, e.g. in small cell carcinoma of the lung.

3. Desmosomes (Macula densa). These are tiny adhesion plates present focally between the adjacent

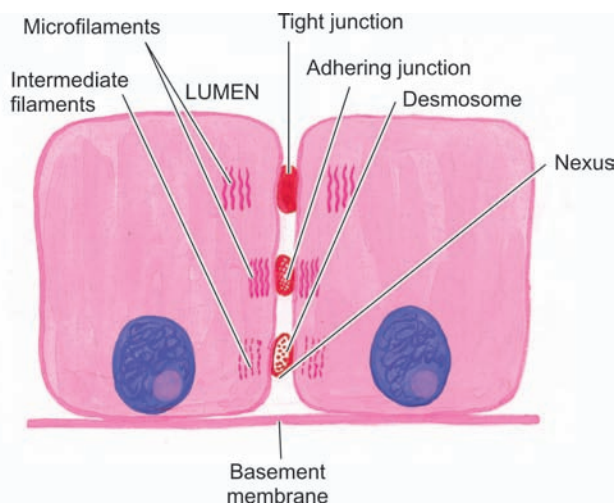


FIGURE 3.3

Intercellular junctions.

epithelial cells, especially numerous in the epidermis. Bundles of intermediate filaments (termed tonofilaments in the case of epidermis) project from the intercellular desmosomes and radiate into the cytoplasm. Hemidesmosomes are a variant of desmosomes, occurring at the basal region of epithelial cells between plasma membrane and the basement membrane.

4. Gap junctions (Nexus). Gap junctions or nexus are the regions on the lateral surfaces of epithelial cells where the gap between the adjoining plasma membranes is reduced from 20 nm to about 2 nm in width. Pits or holes are present in the regions of gap junctions so that these regions are permeable to small tracer particles.

MOLECULAR INTERACTIONS BETWEEN CELLS

All cells in the body, including those in circulation, constantly exchange information with each other. This process is accomplished in the cells by chemical agents, also called as molecular agents or factors. Following categories are common to most cells in the body:

1. Cell adhesion molecules (CAMs)
2. Cytokines
3. Membrane receptors.

1. Cell Adhesion Molecules (CAMs)

These are chemicals which mediate the interaction between cells (cell-cell interaction) as well as between cells and extracellular matrix (cell-ECM interaction). The ECM is the ground substance or matrix of connective

tissue which provides environment to the cells and consists of 3 components:

- i) fibrillar structural proteins (collagen, elastin);
- ii) adhesion proteins (fibronectin, laminin, fibrillin, osteonectin, tenacin); and
- iii) molecules of proteoglycans and glycosaminoglycans (heparan sulphate, chondroitin sulphate, dermatan sulphate, keratan sulphate, hyaluronic acid).

CAMs participate in fertilisation, embryogenesis, tissue repair, haemostasis, cell death by apoptosis and in inflammation. CAMs may be detected on the surface of cells as well as free in circulation. There are 5 groups of CAMs:

i) Integrins: They have alpha (or CD11*) and beta (CD18) subunits and have a role in cell-ECM interactions and in leucocyte-endothelial cell interaction.

ii) Cadherins: These are calcium-dependent adhesion molecules which bind adjacent cells together and prevent invasion of ECM by cancer cells. Various types of cadherins include: E-cadherin (epithelial cell), N-cadherin (nerve cell), M-cadherin (muscle cell), P-cadherin (placenta).

iii) Selectins: Also called as lectins, these CAMs contain lectins or lectin-like protein molecules which bind to glycoproteins and glycolipids on the cell surface. Their major role is in movement of leucocytes and platelets and develop contact with endothelial cells. Selectins are of 3 types: *P-selectin* (from platelets, also called CD62), *E-selectin* (from endothelial cells, also named ECAM), and *L-selectin* (from leucocytes, also called LCAM).

iv) Immunoglobulin superfamily: This group consists of a variety of immunoglobulin molecules present on most cells of the body. These partake in cell-to-cell contact through various other CAMs and cytokines. They have a major role in recognition and binding of immunocompetent cells. This group includes ICAM-1,2 (intercellular adhesion molecule, also called CD54), VCAM (vascular cell adhesion molecule, also named CD106), NCAM (neural cell adhesion molecule).

v) CD44: The last group of adhesion molecules is a break away from immunoglobulin superfamily. CD44 molecule binds to hyaluronic acid and is expressed on leucocytes. It is involved in leucocyte-leucocyte-endothelial interactions as well as in cell-ECM interactions.

2. Cytokines

These are soluble proteins secreted by haemopoietic and non-haemopoietic cells in response to various stimuli.

*CD number (for Cluster of Differentiation) is the nomenclature given to the clone of cells which carry these molecules on their cell surface or in their cytoplasm.

Their main role is in activation of immune system. Presently, about 50 cytokines have been identified which are grouped in 6 categories:

- i) interferons (IFN),
- ii) interleukins (IL),
- iii) tumour necrosis factor (TNF, cachectin),
- iv) transforming growth factor (TGF),
- v) colony stimulating factor (CSF), and
- vi) growth factors.

The examples of growth factors are platelet-derived growth factor (PDGF), epidermal growth factor (EGF), fibroblast growth factor (FGF), endothelial-derived growth factor (EDGF), transforming growth factor (TGF).

All these cytokines have further subtypes as alpha, beta, or are identified by numbers. Cytokines involved in leucocyte-endothelial cell interaction are called *chemokines* while growth factors and other cytokines are named *crinopectins*.

3. Cell Membrane Receptors

Cell receptors are molecules consisting of proteins, glycoproteins or lipoproteins and may be located on the outer cell membrane, inside the cell, or may be trans-membranous. These receptor molecules are synthesised by the cell itself depending upon their requirement, and thus there may be upregulation or downregulation of number of receptors. There are 3 main types of receptors:

i) Enzyme-linked receptors: These receptors are involved in control of cell growth e.g. tyrosine kinase associated receptors take part in activation of synthesis and secretion of various hormones.

ii) Ion channels: The activated receptor for ion exchange such as for sodium, potassium and calcium and certain peptide hormones determines inward or outward movement of these molecules.

iii) G-protein receptors: These are trans-membranous receptors and activate phosphorylating enzymes for metabolic and synthetic functions of cells. The activation of adenosine monophosphate-phosphatase cycle (c-AMP) by the G-proteins (guanosine nucleotide binding regulatory proteins) is the most important signal system, also known as 'second messenger' activation. The activated second messenger (cyclic-AMP) then regulates other intracellular activities.

CELL CYCLE

Multiplication of the somatic (mitosis) and germ (meiosis) cells is the most complex of all cell functions.

Mitosis is controlled by genes which encode for release of specific proteins molecules that promote or inhibit the process of mitosis at different steps. Mitosis promoting protein molecules are *cyclins A, B and E*. These cyclins activate *cyclin-dependent kinases (CDKs)* which act in conjunction with cyclins. After the mitosis is complete, cyclins and CDKs are degraded and the residues of used molecules are taken up by cytoplasmic caretaker proteins, *ubiquitin*, to the peroxisome for further degradation.

Period between the mitosis is called *interphase*. The *cell cycle* is the phase between two consecutive divisions (Fig. 3.4). There are 4 sequential phases in the cell cycle: G_1 (gap 1) phase, S (synthesis) phase, G_2 (gap 2) phase, and M (mitotic) phase.

G_1 (Pre-mitotic gap) phase is the stage when messenger RNAs for the proteins and the proteins themselves required for DNA synthesis (e.g. DNA polymerase) are synthesised. The process is under control of cyclin E and CDKs.

S phase involves replication of nuclear DNA. Cyclin A and CDKs control it.

G_2 (Pre-mitotic gap) phase is the short gap phase in which correctness of DNA synthesised is assessed. This stage is promoted by cyclin B and CDKs.

M phase is the stage in which process of mitosis to form two daughter cells is completed. This occurs in 4 sequential stages: *prophase*, *metaphase*, *anaphase*, and *telophase* (i.e. PMAT).

■ *Prophase*. Each chromosome divides into 2 chromatids which are held together by centromere. The centriole divides and the two daughter centrioles move towards opposite poles of the nucleus and the nuclear membrane disintegrates.

■ *Metaphase*. The microtubules become arranged between the two centrioles forming spindle, while the chromosomes line up at the equatorial plate of the spindle.

■ *Anaphase*. The centromeres divide and each set of separated chromosomes moves towards the opposite poles of the spindle. Cell membrane also begins to divide.

■ *Telophase*. There is formation of nuclear membrane around each set of chromosomes and reconstitution of the nucleus. The cytoplasm of the two daughter cells completely separates.

G_0 phase. The daughter cells may continue to remain in the cell cycle and divide further, or may go out of the cell cycle in resting phase, called G_0 phase.

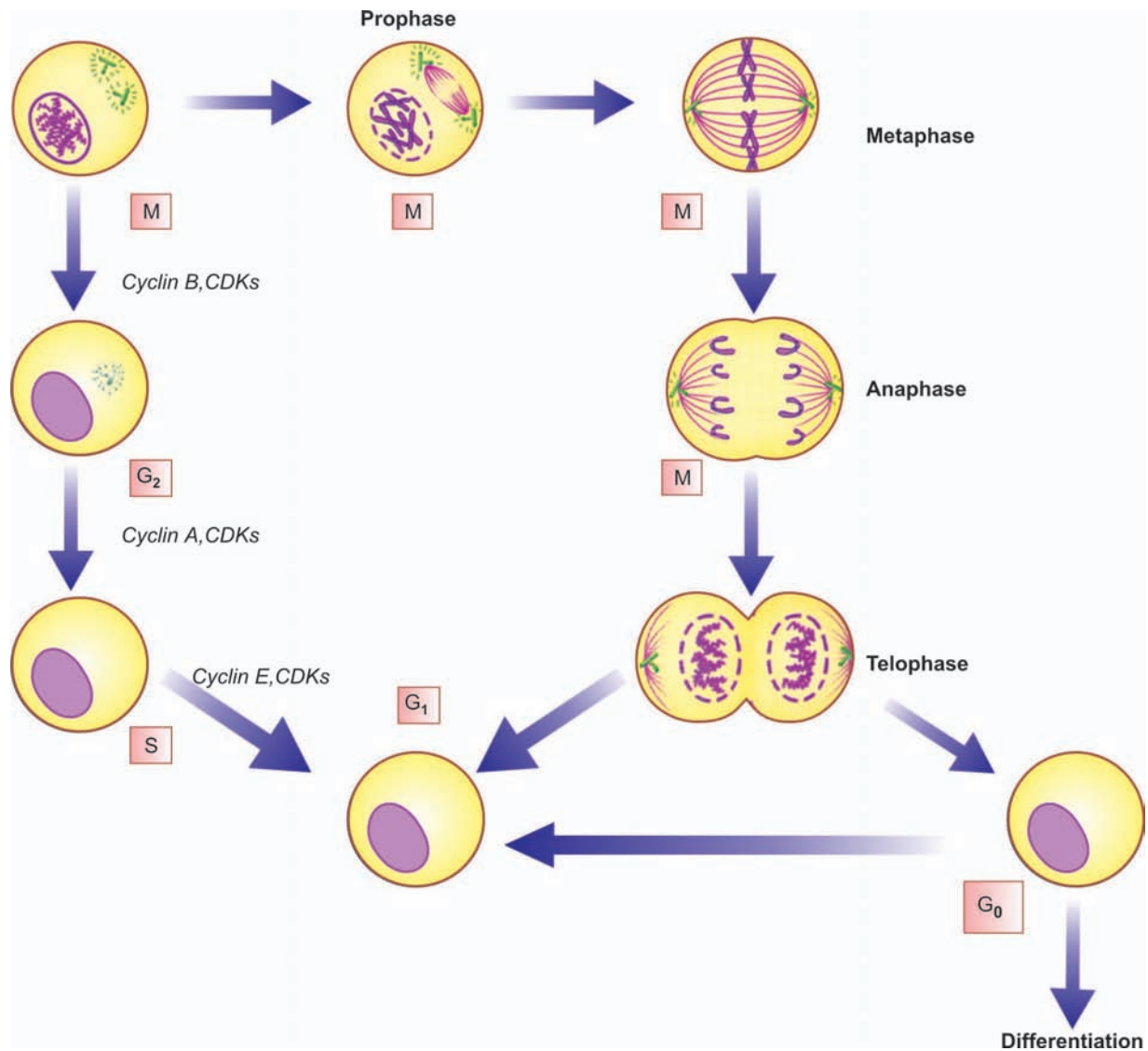


FIGURE 3.4

The cell cycle in mitosis. Premitotic phases are the G_1 , S and G_2 phase while M (mitotic) phase is accomplished in 4 sequential stages: prophase, metaphase, anaphase, and telophase. On completion of cell division, two daughter cells are formed which may continue to remain in the cell cycle or go out of it in resting phase, the G_0 phase. (CDK = cyclin-dependent kinase).

Stimulation of mitosis can be studied in a number of ways as under:

■ *Compensatory stimulation* of mitosis by removal of part of an organ.

■ *Reparative stimulation* of mitosis occurs when a tissue is injured.

■ *Target organ stimulation* of mitosis occurs under the influence of specific hormones which have mitogenic effect on cells of the target organ.



Most forms of diseases begin with cell injury and consequent loss of cellular function. *Cell injury is defined as a variety of stresses a cell encounters as a result of changes in its internal and external environment.* All cells of the body have inbuilt mechanism to deal with changes in environment to an extent. The cellular response to stress varies and depends upon the following variables:

- The type of cell and tissue involved.
- Extent and type of cell injury.

Cellular responses to cell injury may be as follows (Fig. 4.1):

- When there is increased functional demand, the cell may adapt to the changes which are expressed morphologically and then revert back to normal after the stress is removed (*cellular adaptations*; Chapter 19).
- When the stress is mild to moderate, the injured cell may recover (*reversible cell injury*), while when the injury is persistent the cell may die (*irreversible cell injury*).
- The residual effects of reversible cell injury may persist in the cell as evidence of cell injury at subcellular level (*subcellular changes*), or metabolites may accumulate within the cell (*intracellular accumulations*; Chapter 6).

ETIOLOGY OF CELL INJURY

The causes of cell injury, reversible or irreversible, may be broadly classified into two large groups:

- Genetic causes.
- Acquired causes.

The genetic causes of various diseases are discussed in Chapter 8. The acquired causes of disease comprise vast majority of common diseases afflicting mankind. Based on underlying agent, the acquired causes of cell injury can be further categorised as under:

- Hypoxia and ischaemia
- Physical agents
- Chemical agents and drugs
- Microbial agents
- Immunologic agents
- Nutritional derangements
- Psychological factors.

In a given situation, more than one of the above etiologic factors may be involved.

1. HYPOXIA AND ISCHAEMIA. Cells of different tissues essentially require oxygen to generate energy and perform metabolic functions. Deficiency of oxygen or hypoxia results in failure to carry out these activities by the cells. Hypoxia is the most common cause of cell injury. The causes of hypoxia are as under:

- The most common mechanism of hypoxic cell injury is by reduced supply of blood to cells i.e. ischaemia.
- However, oxygen deprivation of tissues may result from other causes as well e.g. in anaemia, carbon

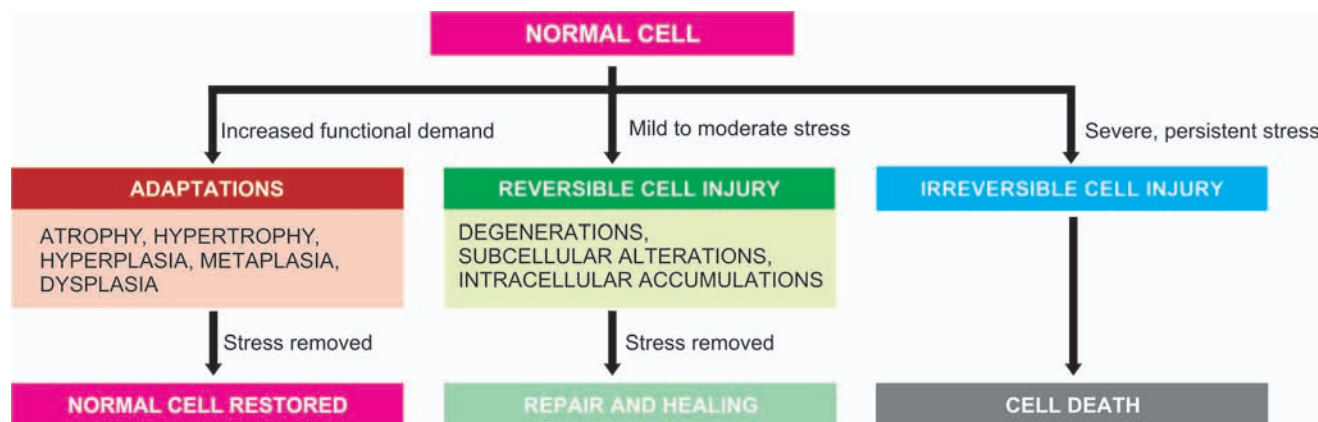


FIGURE 4.1

Cellular responses to cell injury.

monoxide poisoning, cardiorespiratory insufficiency, and increased demand of tissues.

2. PHYSICAL AGENTS. Physical agents in causation of disease are:

- mechanical trauma (e.g. road accidents);
- thermal trauma (e.g. by heat and cold);
- electricity;
- radiation (e.g. ultraviolet and ionising); and
- rapid changes in atmospheric pressure.

3. CHEMICALS AND DRUGS. An ever increasing list of chemical agents and drugs may cause cell injury. Important examples include:

- chemical poisons such as cyanide, arsenic, mercury;
- strong acids and alkalis;
- environmental pollutants;
- insecticides and pesticides;
- oxygen at high concentrations;
- hypertonic glucose and salt;
- social agents such as alcohol and narcotic drugs; and
- therapeutic administration of drugs.

4. MICROBIAL AGENTS. Injuries by microbes include infections caused by bacteria, rickettsiae, viruses, fungi, protozoa, metazoa, and other parasites.

Diseases caused by biologic agents are discussed in Chapter 14.

5. IMMUNOLOGIC AGENTS. Immunity is a 'double-edged sword'—it protects the host against various injurious agents but it may also turn lethal and cause cell injury e.g.

- hypersensitivity reactions;
- anaphylactic reactions; and
- autoimmune diseases.

Immunologic diseases are discussed in Chapter 13.

6. NUTRITIONAL DERANGEMENTS. A deficiency or an excess of nutrients may result in nutritional imbalances.

- Nutritional deficiency diseases may be due to overall deficiency of nutrients (e.g. starvation), of protein calorie (e.g. marasmus, kwashiorkor), of minerals (e.g. anaemia), or of trace elements.
- Nutritional excess is a problem of affluent societies resulting in obesity, atherosclerosis, heart disease and hypertension.

Nutritional diseases are discussed in Chapter 9.

7. PSYCHOLOGICAL FACTORS. There are no specific biochemical or morphologic changes in common acquired mental diseases due to mental stress, strain,

anxiety, overwork and frustration e.g. depression, schizophrenia. However, problems of drug addiction, alcoholism, and smoking result in various organic diseases such as liver damage, chronic bronchitis, lung cancer, peptic ulcer, hypertension, ischaemic heart disease etc.

PATHOGENESIS OF CELL INJURY

The underlying alterations in biochemical systems of cells for reversible and irreversible cell injury by various agents listed above is complex and varied. However, in general, the following principles apply in pathogenesis of most forms of cell injury by various agents:

1. Type, duration and severity of injurious agent: The extent of cellular injury depends upon type, duration and severity of the stimulus e.g. small dose of chemical toxin or short duration of ischaemia cause reversible cell injury while large dose of the same chemical agent or persistent ischaemia cause cell death.

2. Type, status and adaptability of target cell: The type of cell as regards its susceptibility to injury, its nutritional and metabolic status, and adaptation of the cell to hostile environment determine the extent of cell injury e.g. skeletal muscle can withstand hypoxic injury for long time while cardiac muscle suffers irreversible cell injury after 30-60 minutes of persistent ischaemia.

3. Underlying intracellular phenomena: Irrespective of other factors, two essential biochemical phenomena underlie all forms of cell injury to distinguish between reversible and irreversible cell injury:

- i) Inability to reverse mitochondrial dysfunction by reperfusion or reoxygenation.
- ii) Disturbance in membrane function in general, and in plasma membrane in particular.

4. Morphologic consequences: All forms of biochemical changes underlying cell injury are expressed in terms of morphologic changes. The ultrastructural changes become apparent earlier than the light microscopic alterations. The morphologic changes of reversible cell injury (e.g. hydropic swelling) appear earlier than morphologic alterations in cell death (e.g. in myocardial infarction).

The interruption of blood supply (ischaemia) and impaired oxygen supply to the tissues (hypoxia) are most common form of cell injury in human beings. Pathogenesis of hypoxic and ischaemic cell injury is, therefore, discussed in detail below followed by brief discussion on pathogenesis of chemical and physical (ionising radiation) agents. Immunologic tissue injury (Chapter 13) and nutritional factors (Chapter 9) are discussed in respective chapters.

Pathogenesis of Ischaemic and Hypoxic Injury

Ischaemia and hypoxia are common causes of cell injury. The underlying intracellular processes and mechanisms involved in reversible and irreversible cell injury by hypoxia and ischaemia are as under:

REVERSIBLE CELL INJURY. If the ischaemia or hypoxia is of short duration, the effects are reversible on rapid restoration of circulation e.g. in coronary artery occlusion, myocardial contractility, metabolism and ultrastructure are reversed if the circulation is quickly restored. The sequential changes in reversible cell injury are as under (Fig. 4.2):

1. Decreased generation of cellular ATP. ATP is essentially required for a variety of cellular functions such as membrane transport, protein synthesis, lipid synthesis and phospholipid metabolism. ATP in human cell is derived from 2 sources—*firstly*, by aerobic respiration or oxidative phosphorylation (which requires oxygen) in the mitochondria, and *secondly*, by anaerobic glycolytic pathway (in which ATP is generated from glucose/glycogen in the absence of oxygen). Ischaemia and hypoxia both limit the supply of oxygen to the cells, thus causing decreased ATP generation from ADP. But in *ischaemia*, aerobic respiration as well as glucose availability are both compromised resulting in more severe effects of cell injury. On the other hand, in *hypoxia* anaerobic glycolytic energy production continues and thus cell injury is less severe. Secondly, highly specialised cells such as myocardium, proximal tubular cells of the kidney, and neurons of the CNS are dependent on aerobic respiration for ATP generation and thus these tissues suffer from ill-effects of ischaemia more severely and rapidly.

2. Reduced intracellular pH. Due to low oxygen supply to the cell, aerobic respiration by mitochondria fails first. This is followed by switch to anaerobic glycolytic pathway for the energy (i.e. ATP) requirement. This results in rapid depletion of glycogen and accumulation of lactic acid lowering the intracellular pH. Early fall in intracellular pH (i.e. intracellular acidosis) results in clumping of nuclear chromatin.

3. Damage to plasma membrane sodium pump. Normally, the energy (ATP)-dependent sodium pump ($\text{Na}^+ - \text{K}^+$ ATPase) operating at the plasma membrane allows active transport of sodium out of the cell and diffusion of potassium into the cell. Lowered ATP in the cell and consequent increased ATPase activity interfere with this membrane-regulated process. This results in intracellular accumulation of sodium and diffusion of potassium out of cell. The accumulation of sodium in

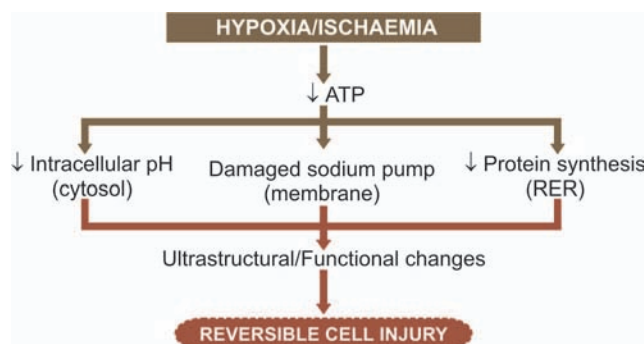


FIGURE 4.2

Sequence of events in the pathogenesis of reversible cell injury caused by hypoxia/ischaemia.

the cell leads to increase in intracellular water to maintain iso-osmotic conditions (hydropic swelling, discussed in the next chapter).

4. Reduced protein synthesis. As a result of continued hypoxia, ribosomes are detached from granular endoplasmic reticulum and polysomes are degraded to monosomes, thus causing reduced protein synthesis.

5. Functional consequences. Reversible cell injury may result in functional disturbances e.g. myocardial contractility ceases within 60 seconds of coronary occlusion but can be reversed if circulation is restored.

6. Ultrastructural changes. Reversible injury to the cell causes the following ultrastructural changes (Fig. 4.3,A):

- i) *Endoplasmic reticulum*: Distension of cisternae by fluid and detachment of membrane-bound polyribosomes from the surface of RER.
- ii) *Mitochondria*: Mitochondrial swelling and phospholipid-rich amorphous densities.
- iii) *Plasma membrane*: Loss of microvilli and focal projections of the cytoplasm ('blebs').
- iv) *Myelin figures*: These are structures lying in the cytoplasm or present outside the cell. They are derived from membranes (plasma or organellar) enclosing water and dissociated lipoproteins between the lamellae of injured membranes.
- v) *Nucleolus*: There is segregation of granular and fibrillar components of nucleolus and reduced synthesis of ribosomal RNA.

Upto this point, withdrawal of acute stress that resulted in reversible cell injury can restore the cell to normal state.

IRREVERSIBLE CELL INJURY. Persistence of ischaemia or hypoxia results in irreversible changes in structure and function of the cell (cell death). The stage

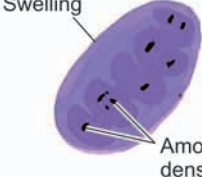
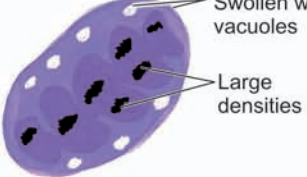
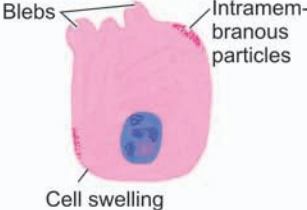
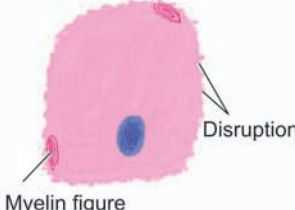
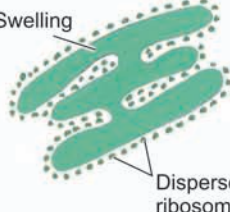
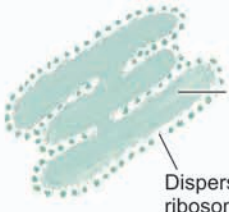
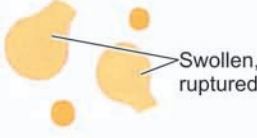
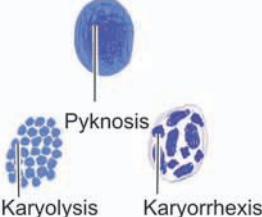
ORGANELLES IN NORMAL CELL	A, REVERSIBLE CELL INJURY	B, IRREVERSIBLE CELL INJURY
 1. MITOCHONDRIA	 Swelling Amorphous densities	 Swollen with vacuoles Large densities
 2. MEMBRANES	 Blebs Intramembranous particles Cell swelling	 Disruption Myelin figure
 3. RER & RIBOSOMES	 Swelling Dispersed ribosomes	 Lysed Dispersed ribosomes
 4. LYSOSOMES	 Autophagy	 Swollen, ruptured
 5. CYTOSKELETON	 Aggregated	 Disrupted
 6. NUCLEUS	 Clumped chromatin	 Pyknosis Karyolysis Karyorrhexis

FIGURE 4.3

Ultrastructural changes during cell injury due to hypoxia-ischaemia.

at which this *point of no return* is reached from reversible cell injury is unclear but the sequence of events is a continuation of reversibly injured cell. Two essential phenomena always distinguish irreversible from reversible cell injury (Fig. 4.3,B):

- Inability of the cell to reverse *mitochondrial dysfunction* on reperfusion or reoxygenation; and
- *Disturbance in cell membrane function* in general, and in plasma membrane in particular.

In addition, there is continued depletion of proteins, leakage of lysosomal enzymes into the cytoplasm, reduced intracellular pH and further reduction in ATP.

1. Mitochondrial dysfunction. As a result of continued hypoxia, a large cytosolic influx of Ca^{++} ions occurs, especially after reperfusion of irreversibly injured cell, which is taken up by mitochondria and is the cause of mitochondrial dysfunction. Morphologically, mitochondrial changes seen are vacuoles in the mitochondria and deposits of amorphous calcium salts in the mitochondrial matrix.

2. Membrane damage. Defect in membrane function in general, and plasma membrane in particular, is the most important event in irreversible cell injury in ischaemia. The mechanisms underlying membrane damage are as under (Fig. 4.4):

- i) *Accelerated degradation of membrane phospholipid.* Oxygen deprivation causes shift of calcium from mitochondria and endoplasmic reticulum into the cytosol. Increased level of calcium in the cytosol activates endogenous phospholipases from ischaemic tissue which degrade membrane phospholipids progressively which are the main constituent of the lipid bilayer membrane. An alternate hypothesis is decreased replacement-synthesis of membrane phospholipids due to reduced ATP.
- ii) *Cytoskeletal damage.* The normal intermediate filaments of the cytoskeleton which are anchored to the cell membrane are damaged due to degradation by activated intracellular proteases or by physical effect of cell swelling producing irreversible cell membrane injury.
- iii) *Toxic oxygen radicals.* Another important mechanism of irreversible cell injury is free-radical induced (described below). Reactive oxygen-derived species—superoxide (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($\text{OH}^\cdot$), are increased in ischaemia. These oxygen radicals are produced during reperfusion by incoming polymorphs. Free radical injury is operative not only in hypoxia but also in radiation injury, chemical injury, microbial killing, aging, atherosclerosis, carcinogenesis.

iv) *Break down products of lipid.* The lipid break down products and catabolic products which accumulate in the injured cell cause further damage to various cell membranes.

v) *Reperfusion damage.* Normally, the concentration of calcium ions in extracellular fluid is 10^{-3} M while that in the cytosol is much low 10^{-7} M (M for millimole). Upon reperfusion of irreversibly injured cell, calcium homeostasis is disturbed and there is large cytosolic influx of calcium ions. An increased cytosolic calcium concentration may activate membrane phospholipases leading to phospholipid degradation, or calcium-dependent proteases may get activated to bring about membrane damage.

3. Hydrolytic enzymes. Damage to lysosomal membranes is followed by liberation of *hydrolytic enzymes* (RNAase, DNAase, proteases, glycosidases, phosphatases, and cathepsin) which on activation cause enzymatic digestion of cellular components and induce the nuclear changes (pyknosis, karyolysis and karyorrhexis) and hence cell death. The dead cell is eventually replaced by masses of phospholipids called *myelin figures* which are either phagocytosed by macrophages or there may be formation of calcium soaps.

4. Serum estimation of liberated intracellular enzymes. Liberated enzymes just mentioned leak across the abnormally permeable cell membrane into the serum, the estimation of which may be used as clinical parameters of cell death. For example, in myocardial infarction, estimation of elevated serum glutamic oxaloacetic transaminase (SGOT), lactic dehydrogenase (LDH), isoenzyme of creatine kinase (CK-MB), and cardiac troponins (cTn) are useful guides for death of heart muscle.

Ischaemia-Reperfusion Injury

Depending upon the duration of ischaemia/hypoxia, restoration of blood flow may result in the following 3 different consequences:

1. *When the period of ischaemia is of short duration*, reperfusion with resupply of oxygen restores the structural and functional state of the injured cell i.e. reversible cell injury.
2. *When ischaemia is for longer duration*, then rather than restoration of structure and function of the cell, reperfusion paradoxically deteriorates the already injured cell. This is termed ischaemia-reperfusion injury. The main proposed mechanism in ischaemia-reperfusion injury is that upon reoxygenation there is increased generation of oxygen free radicals or activated oxygen species (superoxide, H_2O_2 , hydroxyl radicals) from incoming

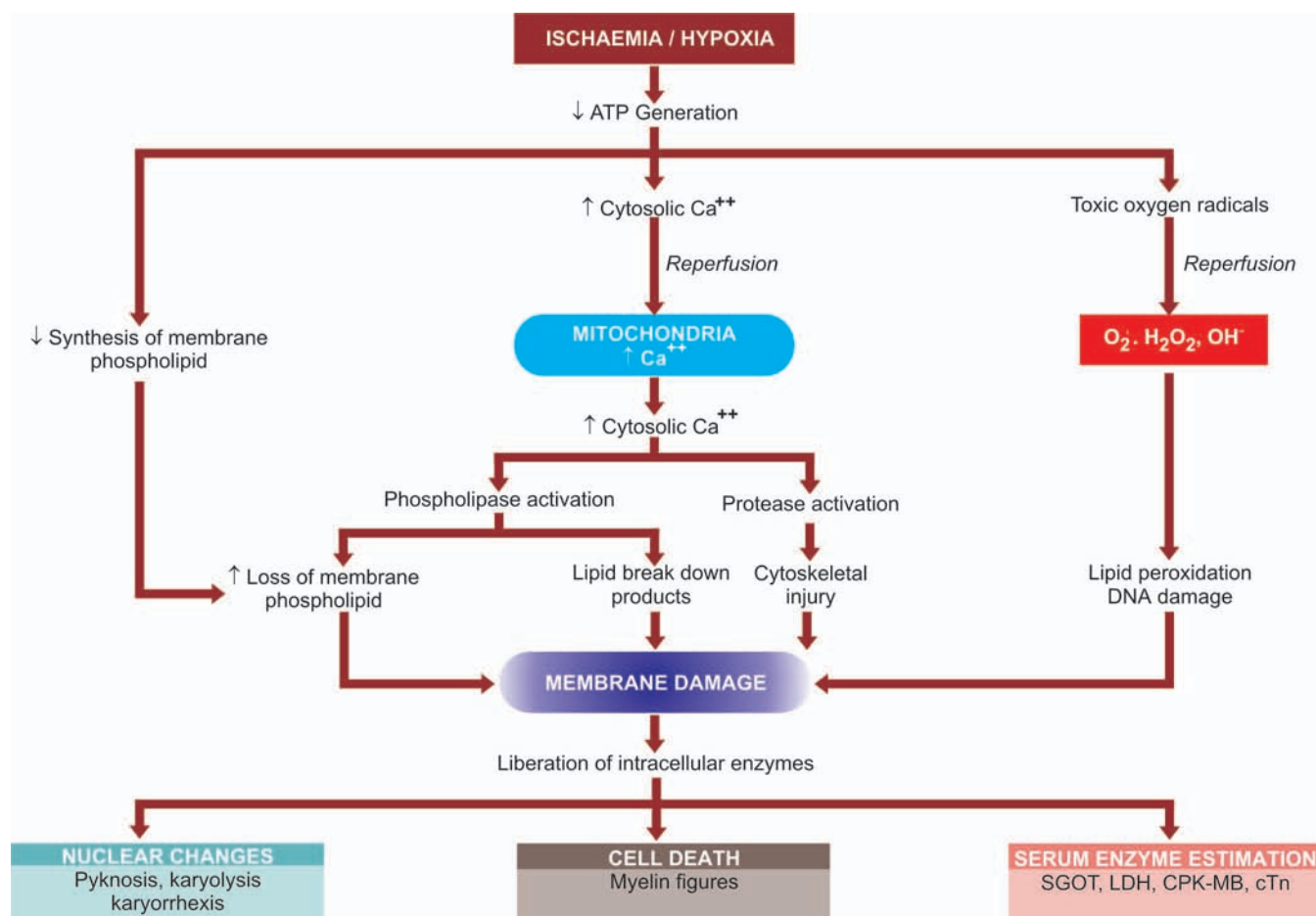


FIGURE 4.4

Possible sequence of events in the pathogenesis of irreversible cell injury in ischaemia.

inflammatory cells. Alternatively, during reperfusion activated oxygen species may be generated by adhesion and activation of circulating neutrophils. Besides, ischaemia also damages the cellular antioxidant defense mechanism favouring further accumulation of oxygen free radicals.

3. *Longer period of ischaemia* may also produce irreversible cell injury *during ischaemia itself* without any role of reperfusion. Cell death in such cases is not attributed to formation of activated oxygen species. But instead on reperfusion there is marked intracellular excess of sodium and calcium ions due to cell membrane damage.

Free Radical-Mediated Cell Injury

Although oxygen is the lifeline of all cells and tissues, its molecular forms as oxygen free radicals (or reactive

oxygen intermediates) can be most devastating for the cells. Free radical-mediated cell injury plays an important role in the following situations:

- i) In ischaemic reperfusion injury (as mentioned above in mechanism of membrane damage in hypoxia)
- ii) In ionising radiation by causing radiolysis of water
- iii) In chemical toxicity
- iv) Hyperoxia (toxicity due to oxygen therapy)
- v) Cellular aging
- vi) Killing of exogenous biologic agents
- vii) Inflammatory damage
- viii) Destruction of tumour cells
- ix) Chemical carcinogenesis
- x) Atherosclerosis.

Generation of oxygen free radicals begins within mitochondrial inner membrane when cytochrome oxidase catalyses the four electron reduction of oxygen

(O_2) to water (H_2O). Intermediate between reaction of O_2 to H_2O , three partially reduced species of oxygen are generated depending upon the number of electrons transferred. These are:

- Superoxide oxygen (O_2^-): one electron
- Hydrogen peroxide (H_2O_2): two electrons
- Hydroxyl radical ($OH^\cdot$): three electrons.

A few other oxygen radicals which may be generated in reactions other than those during O_2 to H_2O are: hypochlorous acid ($HOCl$), peroxyxynitrate ion ($ONOO^-$), nitric oxide (NO) generated by various body cells (endothelial cells, neurons, macrophages etc), and release of superoxide free radical in Fenton reaction (see below).

FREE RADICAL GENERATION. The three partially reduced intermediate species between O_2 to H_2O are derived from enzymatic and non-enzymatic reaction as under (Fig. 4.5):

1. *Superoxide (O_2^-):* Superoxide anion O_2^- may be generated by direct auto-oxidation of O_2 during mitochondrial electron transport reaction. Alternatively, O_2^- is produced enzymatically by xanthine oxidase and cytochrome P_{450} in the mitochondria or cytosol. O_2^- so formed is catabolised to produce H_2O_2 by superoxide dismutase (SOD).
2. *Hydrogen peroxide (H_2O_2):* H_2O_2 is reduced to water enzymatically by catalase (in the peroxisomes) and glutathione peroxidase GSH (both in the cytosol and mitochondria).

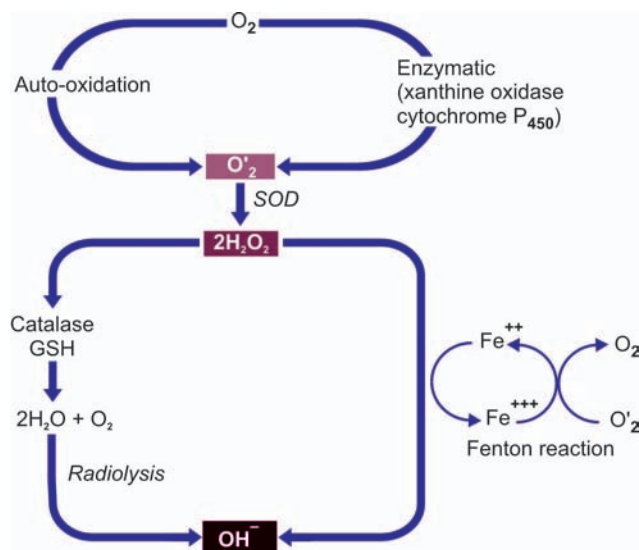


FIGURE 4.5

Mechanisms of generation of free radicals by free radicals. (SOD = superoxide dismutase; GSH = glutathione peroxidase).

3. *Hydroxyl radical ($OH^\cdot$):* $OH^\cdot$ radical is formed by 2 ways in biologic processes: by radiolysis of water and by reaction of H_2O_2 with ferrous (Fe^{++}) ions; the latter process is termed as Fenton reaction.

FREE RADICAL REACTIONS. The hydroxyl radical is the most reactive species. It may produce membrane damage by the following mechanisms (Fig. 4.6):

- i) *Lipid peroxidation.* Polyunsaturated fatty acids (PUFA) of membrane are attacked repeatedly and severely by oxygen-derived free radicals to yield highly destructive PUFA radicals—lipid hydroperoxy radicals and lipid hypoperoxides. This reaction is termed lipid peroxidation. The lipid peroxides are decomposed by transition metals such as iron. Lipid peroxidation is propagated to other sites causing widespread membrane damage and destruction of organelles.
- ii) *Oxidation of proteins.* Oxygen-derived free radicals cause cell injury by oxidation of protein macromolecules of the cells, cross linking of labile amino acids as well as by fragmentation of polypeptides directly. The end-result is degradation of cytosolic neutral proteases and cell destruction.
- iii) *DNA damage.* Free radicals cause breaks in the single strands of the nuclear and mitochondrial DNA. This results in cell injury; it may also cause malignant transformation of cells.
- iv) *Cytoskeletal damage.* Reactive oxygen species are also known to interact with cytoskeletal elements and interfere in mitochondrial aerobic phosphorylation and thus cause ATP depletion.

ANTI-OXIDANTS. Anti-oxidants are endogenous or exogenous substances which inactivate the free radicals. These substances include:

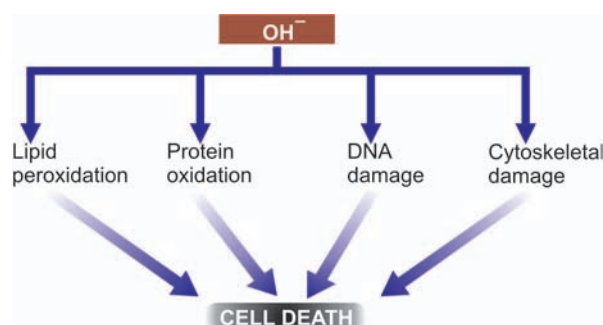


FIGURE 4.6

Mechanism of cell death by hydroxyl radical, the most reactive oxygen species.

- Vitamins E, A and C (ascorbic acid)
- Sulfhydryl-containing compounds e.g. cysteine and glutathione.
- Serum proteins e.g. ceruloplasmin and transferrin.

Free radicals are formed in physiologic as well as pathologic processes. However, oxygen radicals are basically unstable and are destroyed spontaneously. The rate of spontaneous destruction is determined by catalytic action of certain enzymes such as superoxide dismutase (SOD), catalase and glutathione peroxidase. The net effect of free radical injury in physiologic and disease states, therefore, depends upon the rate of free radical formation and rate of their elimination.

Pathogenesis of Chemical Injury

Chemicals induce cell injury by one of the following two mechanisms:

DIRECT CYTOTOXIC EFFECTS. Some chemicals combine with components of the cell and produce direct cytotoxicity without requiring metabolic activation. The cytotoxic damage is usually greatest to cells which are involved in the metabolism of such chemicals e.g. in mercuric chloride poisoning, the greatest damage occurs to cells of the alimentary tract and kidney. Cyanide kills the cell by poisoning mitochondrial cytochrome oxidase thus blocking oxidative phosphorylation.

Examples of directly cytotoxic chemicals include chemotherapeutic agents used in treatment of cancer, toxic heavy metals such as mercury, lead and iron.

CONVERSION TO REACTIVE TOXIC METABOLITES. This mechanism involves metabolic activation to yield ultimate toxin that interacts with the target cells. The target cells in this group of chemicals may not be the same cell that metabolised the toxin. Example of cell injury by conversion of reactive metabolites is toxic liver necrosis caused by carbon tetrachloride (CCl_4), acetaminophen (commonly used analgesic and antipyretic) and bromobenzene. Cell injury by CCl_4 is classic example of an industrial toxin (used in dry-cleaning industry) that produces cell injury by products produced from the body's drug-metabolising P_{450} enzyme system. The underlying mechanisms include free radical injury, and direct toxic effect on cell membrane and nucleus.

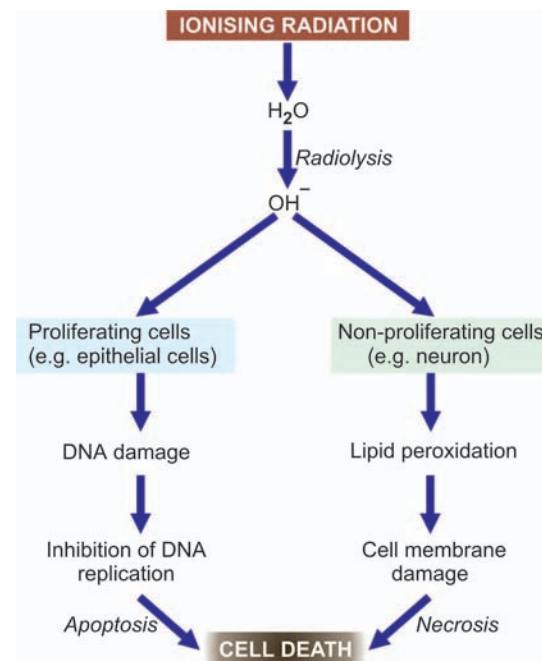


FIGURE 4.7

Mechanisms of cell injury by ionising radiation.

Pathogenesis of Physical Injury

Injuries caused by mechanical force are of medicolegal significance. But they may lead to a state of shock. Injuries by changes in atmospheric pressure (e.g. decompression sickness) are detailed in Chapter 17. Radiation injury to human by accidental or therapeutic exposure is of importance in treatment of persons with malignant tumours as well as may have carcinogenic influences (Chapter 21).

Killing of cells by *ionising radiation* is the result of direct formation of hydroxyl radicals from radiolysis of water (Fig. 4.7). These hydroxyl radicals damage the cell membrane as well as may interact with DNA of the target cell. In proliferating cells, there is inhibition of DNA replication and eventual cell death by apoptosis (e.g. epithelial cells). In non-proliferating cells there is no effect of inhibition of DNA synthesis and in these cells there is cell membrane damage followed by cell death by necrosis (e.g. neurons).



After having discussed the molecular and biochemical mechanisms of various forms of cell injury, we now turn to light microscopic morphologic changes of reversible and irreversible cell injury.

Depending upon the severity of cell injury, degree of damage and residual effects on cells and tissues are variable. In general, morphologic changes in various forms of cell injury can be classified as shown in Table 5.1 and are discussed below.

MORPHOLOGY OF REVERSIBLE CELL INJURY

In conventional description of morphologic changes, the term degeneration has been used to denote morphology of reversible cell injury. However, now it is realised that this term does not provide any information on the nature of underlying changes and thus currently more acceptable terms of *retrogressive changes* or simply reversible cell injury are applied to non-lethal cell injury.

Following morphologic forms of reversible cell injury are included under this heading:

1. Cellular swelling (hydropic change, or vacuolar degeneration)
2. Fatty change
3. Hyaline change
4. Muroid change

TABLE 5.1: Classification of Morphologic Forms of Cell Injury.

MECHANISM OF CELL INJURY	NOMENCLATURE
1. <i>Reversible cell injury</i>	Retrogressive changes (older term: degenerations)
2. <i>Irreversible cell injury</i>	Cell death—necrosis
3. <i>Programmed cell death</i>	Apoptosis
4. <i>Residual effects of cell injury</i>	Subcellular alterations
5. <i>Deranged cell metabolism</i>	Intracellular accumulation of lipid, protein, carbohydrate
6. <i>After-effects of necrosis</i>	Gangrene, pathologic calcification

Cellular Swelling

This is the commonest and earliest form of cell injury from almost all causes. Other synonyms of cellular swelling used in the past are *cloudy swelling* (for gross appearance of the affected organ), *hydropic change* (accumulation of water within the cell), and *vacuolar degeneration* (due to cytoplasmic vacuolation).

The common causes of cellular swelling include: bacterial toxins, chemicals, poisons, burns, high fever, intravenous administration of hypotonic glucose or saline etc.

Cloudy swelling results from impaired regulation of cellular volume, especially for sodium. This regulation is operative at 3 levels: at the plasma membrane itself, at the sodium pump on the plasma membrane, and at the supply of ATP. Injurious agent may interfere with these regulatory mechanisms and result in accumulation of sodium in the cell which, in turn, leads to inflow of water to maintain iso-osmotic conditions and hence cellular swelling occurs.

Grossly, the affected organ such as kidney, liver or heart muscle is enlarged due to swelling. The cut surface bulges outwards and is slightly opaque.

Microscopically, it is characterised by the following features (Fig. 5.1).

1. The cells are swollen and the microvasculature compressed. The cellular swelling is due to increased influx of sodium and extracellular water into the cell and escape of potassium.
2. Small clear vacuoles are seen in the cells and hence the term vacuolar degeneration. These vacuoles represent distended cisternae of the endoplasmic reticulum.

Ultrastructural changes in hydropic swelling include the following:

- i) Dilatation of endoplasmic reticulum.
- ii) Detachment of polysomes from the surface of RER.
- iii) Mitochondrial swelling.
- iv) Blebs on the plasma membrane.
- v) Loss of fibrillarity of nucleolus.

It may be mentioned here the hydropic swelling is entirely reversible if the injurious agent is removed.

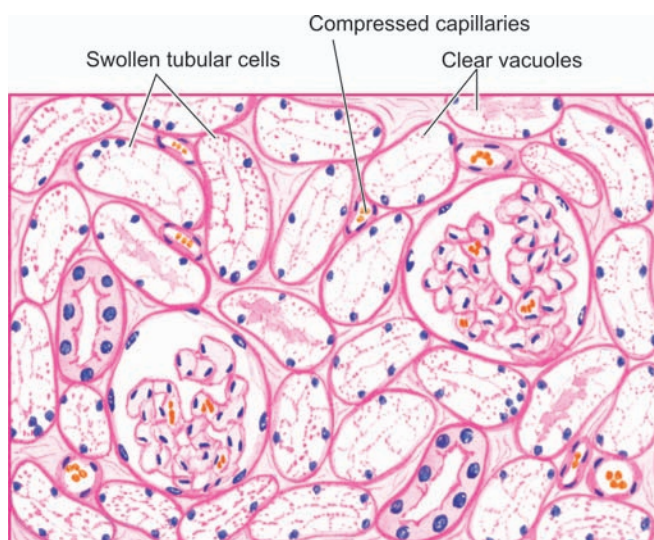


FIGURE 5.1

Hydropic change kidney. The tubular epithelial cells are distended with cytoplasmic vacuoles while the interstitial vasculature is compressed.

Hyaline Change

The word 'hyaline' means glassy (*hyalos* = glass). Hyaline is a descriptive histologic term for glassy, homogeneous, eosinophilic appearance of material in haematoxylin and eosin-stained sections and does not refer to any specific substance. Hyaline change is associated with heterogeneous pathologic conditions and may be intracellular or extracellular.

INTRACELLULAR HYALINE. Intracellular hyaline is mainly seen in epithelial cells. For example:

1. *Hyaline droplets* in the proximal tubular epithelial cells in cases of excessive reabsorption of plasma proteins.
2. *Hyaline degeneration* of voluntary muscle, also called Zenker's degeneration, occurs in rectus abdominalis muscle in typhoid fever. The muscle loses its fibrillar staining and becomes glassy and hyaline.
3. *Mallory's hyaline* represents aggregates of intermediate filaments in the hepatocytes in alcoholic liver cell injury.
4. Nuclear or cytoplasmic *hyaline inclusions* seen in some viral infections.
5. *Russell's bodies* representing excessive immunoglobulins in the rough endoplasmic reticulum of the plasma cells.

EXTRACELLULAR HYALINE. Extracellular hyaline is seen in connective tissues. A few examples of extracellular hyaline change are:

1. Hyaline degeneration in *leiomyomas* of the uterus (**COLOUR PLATE I: CL 1**).
2. Hyalinised *old scar* of fibrocollagenous tissues.
3. *Hyaline arteriosclerosis* in renal vessels in hypertension and diabetes mellitus.
4. Hyalinised glomeruli in *chronic glomerulonephritis*.
5. *Corpora amylacea* are rounded masses of concentric hyaline laminae seen in the prostate in the elderly, in the brain and in the spinal cord in old age, and in old infarcts of the lung.

Though fibrin and amyloid have hyaline appearance, they have distinctive features and staining reactions and can be distinguished from non-specific hyaline material.

Mucoid Change

Mucus secreted by mucous glands is a combination of proteins complexed with mucopolysaccharides. *Mucin*, a glycoprotein, is its chief constituent. Mucin is normally produced by epithelial cells of mucous membranes and mucous glands, as well as by some connective tissues like in the umbilical cord. Epithelial mucin stains positively with periodic acid-Schiff (PAS), while connective tissue mucin is PAS negative but is stained positively with colloidal iron. Both are, however, stained by alcian blue.

EPITHELIAL MUCIN. Some examples of functional excess of epithelial mucin are:

1. Catarrhal inflammation of mucous membrane (e.g. of respiratory tract, alimentary tract, uterus).
2. Obstruction of duct leading to mucocele in the oral cavity and gall bladder.
3. Cystic fibrosis of the pancreas.
4. Mucin-secreting tumours (e.g. of ovary, stomach, large bowel etc).

CONNECTIVE TISSUE MUCIN. A few examples of disturbances of connective tissue mucin are:

1. Mucoid or myxoid degeneration in some tumours e.g. myxomas, neurofibromas soft tissue sarcomas etc.
2. Dissecting aneurysm of aorta due to Erdheim's medial degeneration and Marfan's syndrome.
3. Myxomatous change in the dermis in myxoedema.
4. Myxoid change in the synovium in ganglion on the wrist.

SUBCELLULAR ALTERATIONS IN CELL INJURY

Certain morphologically distinct alterations at subcellular level are noticeable in both acute and chronic forms of cell injury. These occur at the level of cytoskeleton, lysosomes, endoplasmic reticulum and mitochondria:

1. CYTOSKELETAL CHANGES. Components of cytoskeleton may show the following morphologic abnormalities:

i) Defective microtubules:

- In Chédiak-Higashi syndrome characterised by poor phagocytic activity of neutrophils.
- Poor sperm motility causing sterility.
- Immotile cilia syndrome (Kartagener's syndrome) characterised by immotile cilia of respiratory tract and consequent chronic infection due to defective clearance of inhaled bacteria.
- Defects in leucocyte function of phagocytes such as migration and chemotaxis.

ii) Defective microfilaments:

- In myopathies.
- Muscular dystrophies.

iii) Accumulation of intermediate filaments: Various classes of intermediate filaments (cytokeratin, desmin, vimentin, glial fibrillary acidic protein, and neurofilament) may accumulate in the cytosol. For example:

- Mallory's body or alcoholic hyaline as intracytoplasmic eosinophilic inclusion seen in alcoholic liver disease which is collection of cytokeratin intermediate filaments.
- Neurofibrillary tangles, neurites and senile plaques in Alzheimer's disease are composed of neurofilaments and paired helical filaments.

2. LYSOSOMAL CHANGES. Lysosomes contain powerful hydrolytic enzymes. Heterophagy and autophagy are the two ways by which lysosomes show morphologic changes of phagocytic function.

i) Heterophagy. Phagocytosis (cell eating) and pinocytosis (cell drinking) are the two forms by which material from outside is taken up by the lysosomes of cells such as polymorphs and macrophages to form *phagolysosomes*. This is termed heterophagy. Microbial agents and foreign particulate material are eliminated by this mechanism.

ii) Autophagy. This is the process by which worn out intracellular organelles and other cytoplasmic material form autophagic vacuole that fuses with lysosome to form *autophagolysosome*.

iii) Indigestible material. Some indigestible exogenous particles such as carbon or endogenous substances such as lipofuscin may persist in the lysosomes of the cells for a long time as residual bodies.

iv) Storage diseases. As discussed in Chapter 8, a group of lysosomal storage diseases due to hereditary deficiency of enzymes may result in abnormal collection of metabolites in the lysosomes of cells.

3. S.E.R. HYPERTROPHY. Hypertrophy of smooth endoplasmic reticulum of liver cells as an adaptive change may occur in response to prolonged use of barbiturates.

4. MITOCHONDRIAL CHANGES. Mitochondrial injury plays an important role in cell injury. Morphologic changes of cell injury in mitochondria may be seen in the following conditions:

i) *Megamitochondria.* Megamitochondria consisting of unusually big mitochondria are seen in alcoholic liver disease and nutritional deficiency conditions.

ii) Alterations in the *number of mitochondria* may occur. Their number increases in hypertrophy and decreases in atrophy.

iii) *Oncocytoma* in the salivary glands, thyroid and kidneys consists of tumour cells having very large mitochondria.

iv) *Myopathies* having defect in mitochondria have abnormal cristae.

5. HEAT SHOCK PROTEINS AND UBIQUITIN. Heat shock proteins (HSP), more appropriately called *stress proteins*, are a variety of intracellular carrier proteins present in most cells for physiologic function. However, in response to stresses of various types (e.g. toxins, drugs, poisons, ischaemia), their level goes up both inside the cell as well as leak out into the plasma, and hence the name stress proteins. They perform the role of *chaperones* (house-keeping) i.e. they direct and guide metabolic molecules to the sites of metabolic activity e.g. protein folding, disaggregation of protein-protein complexes and transport of proteins into various intracellular organelles (*protein kinesin*).

In experimental studies HSPs have been shown to limit tissue necrosis in ischaemic reperfusion injury in myocardial infarcts. In addition, they have also been shown to have a central role in protein aggregation in amyloidosis.

Another related stress protein, *ubiquitin* (so named due to its ubiquitous or universal presence in the cells of the body), has been found to be involved in activation

of genes for protein synthesis in neurodegenerative diseases such as in Alzheimer's disease, Creutzfeldt-Jakob disease, Parkinson's disease.

MORPHOLOGY OF IRREVERSIBLE CELL INJURY (CELL DEATH)

Cell death is a state of irreversible injury. It may occur in the living body as a local or focal change (i.e. autolysis, necrosis and apoptosis) and the changes that follow it (i.e. gangrene and pathologic calcification), or result in end of the life (somatic death). These pathologic processes involved in cell death are described below.

AUTOLYSIS

Autolysis ('self-digestion') is disintegration of the cell by its own hydrolytic enzymes liberated from lysosomes. Autolysis can occur in the living body when it is surrounded by inflammatory reaction (*vital reaction*), or may occur as postmortem change in which there is complete absence of surrounding inflammatory response. Autolysis is *rapid* in some tissues rich in hydrolytic enzymes such as in the pancreas, and gastric mucosa; *intermediate* in tissues like the heart, liver and kidney; and *slow* in fibrous tissue. Morphologically, autolysis is identified by homogeneous and eosinophilic cytoplasm with loss of cellular details and remains of cell as debris.

NECROSIS

Necrosis is defined as focal death along with degradation of tissue by hydrolytic enzymes liberated by cells. It is invariably accompanied by inflammatory reaction.

Necrosis can be caused by various agents such as hypoxia, chemical and physical agents, microbial agents and immunological injury. Two essential changes bring about irreversible cell injury in necrosis—cell digestion by lytic enzymes and denaturation of proteins. These processes are morphologically identified by characteristic cytoplasmic and nuclear changes in necrotic cell (Fig. 5.2,A). The cytoplasm appears homogeneous and intensely eosinophilic. Occasionally, it may show vacuolation or dystrophic calcification. The nuclear changes include condensation of nuclear chromatin (*pyknosis*) which may either undergo dissolution (*karyolysis*) or fragmentation into many granular clumps (*karyorrhexis*) (see Fig. 4.3, page 28).

Types of Necrosis

Morphologically, 5 distinct types of necrosis are identified: coagulative, liquefaction (colliquative), caseous, fat, and fibrinoid necrosis.

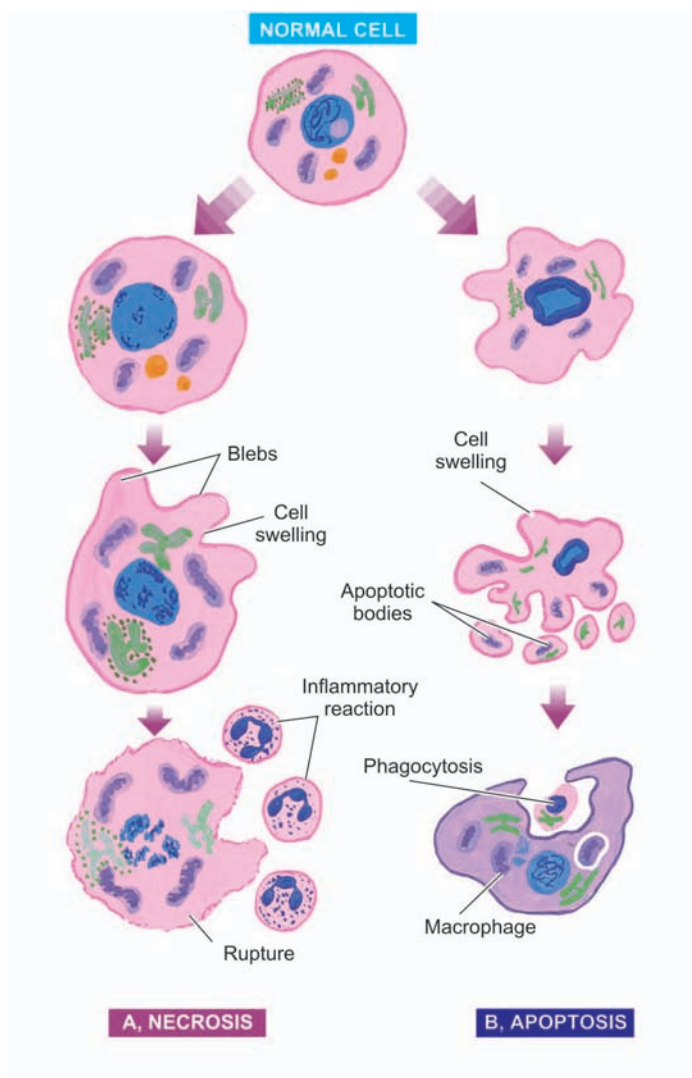


FIGURE 5.2

Necrosis and apoptosis. A, Cell necrosis is identified by homogeneous, eosinophilic cytoplasm and nuclear changes of pyknosis, karyolysis, and karyorrhexis. B, Apoptosis consists of condensation of nuclear chromatin and fragmentation of the cell into membrane-bound apoptotic bodies which are engulfed by macrophages.

1. COAGULATIVE NECROSIS. This is the most common type of necrosis caused by irreversible focal injury, mostly from sudden cessation of blood flow (ischaemia), and less often from bacterial and chemical agents. The organs commonly affected are the heart, kidney, and spleen.

Grossly, foci of coagulative necrosis in the early stage are pale, firm, and slightly swollen. With progression, they become more yellowish, softer, and shrunken.

Microscopically, the hallmark of coagulative necrosis is the conversion of normal cells into their 'tombstones' i.e. outlines of the cells are retained so that the cell type can still be recognised but their cytoplasmic and nuclear details are lost. The necrosed cells are swollen and appear more eosinophilic than the normal, along with nuclear changes described above. This pattern of microscopic change results from 2 processes: denaturation of proteins and enzymatic digestion of the cell. But cell digestion and liquefaction fail to occur (c.f. liquefaction necrosis). Eventually, the necrosed focus is infiltrated by inflammatory cells and the dead cells are phagocytosed leaving granular debris and fragments of cells (Fig. 5.3,A).

2. LIQUEFACTION (COLLIQUATIVE) NECROSIS.

Liquefaction or colliquative necrosis occurs commonly due to ischaemic injury and bacterial or fungal infections. It occurs due to degradation of tissue by the action of powerful hydrolytic enzymes. The common examples are infarct brain and abscess cavity.

Grossly, the affected area is soft with liquefied centre containing necrotic debris. Later, a cyst wall is formed.

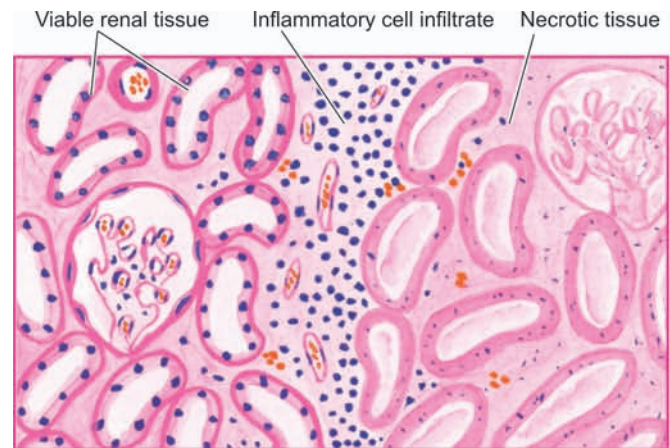
Microscopically, the cystic space contains necrotic cell debris and macrophages filled with phagocytosed material. The cyst wall is formed by proliferating capillaries, inflammatory cells, and gliosis (proliferating glial cells) in the case of brain and proliferating fibroblasts in the case of abscess cavity (Fig. 5.3,B).

3. CASEOUS NECROSIS. Caseous necrosis is found in the centre of foci of tuberculous infections. It combines features of both coagulative and liquefactive necrosis.

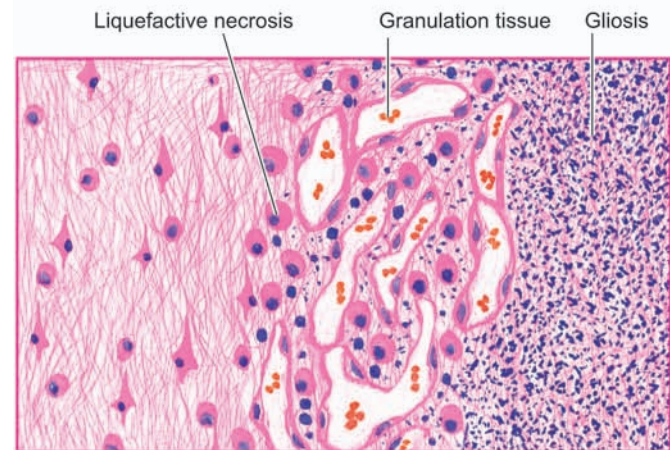
Grossly, foci of caseous necrosis, as the name implies, resemble dry cheese and are soft, granular and yellowish. This appearance is partly attributed to the histotoxic effects of lipopolysaccharides present in the capsule of the tubercle bacilli, *Mycobacterium tuberculosis*.

Microscopically, the necrosed foci are structureless, eosinophilic, and contain granular debris (Fig. 5.3,C). The surrounding tissue shows characteristic granulomatous inflammatory reaction consisting of epithelioid cells with interspersed giant cells of Langhans' or foreign body type and peripheral mantle of lymphocytes (COLOUR PLATE IV: CL 14, 15).

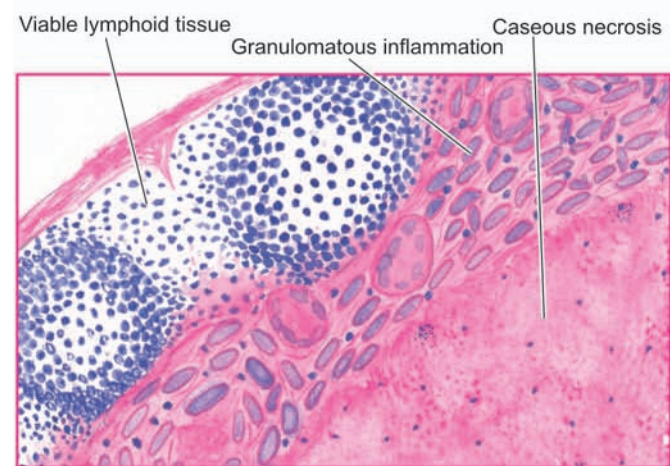
4. FAT NECROSIS. Fat necrosis is a special form of cell death occurring at two anatomically different locations



A, COAGULATIVE NECROSIS (KIDNEY)



B, LIQUEFACTIVE NECROSIS (BRAIN)



C, CASEOUS NECROSIS (LYMPH NODE)

FIGURE 5.3

Principal types of necrosis. (For details, consult the text).

but morphologically similar lesions. These are: following *acute pancreatic necrosis*, and *traumatic fat necrosis* commonly in breasts.

In the case of pancreas, there is liberation of pancreatic lipases from injured or inflamed tissue that results in necrosis of the pancreas as well as of the fat depots throughout the peritoneal cavity, and sometimes, even affecting the extra-abdominal adipose tissue.

Fat necrosis in either of the two instances results in hydrolysis of neutral fat present in adipose cells into glycerol and free fatty acids. The damaged adipose cells assume cloudy appearance when only free fatty acids remain behind, after glycerol leaks out. The leaked out free fatty acids, on the other hand, complex with calcium to form calcium soaps (saponification) discussed later under dystrophic calcification.

Grossly, fat necrosis appears as yellowish-white and firm deposits. Formation of calcium soaps imparts the necrosed foci firmer and chalky white appearance.

Microscopically, the necrosed fat cells have cloudy appearance and are surrounded by an inflammatory reaction. Formation of calcium soaps is identified in the tissue sections as amorphous, granular and basophilic material.

5. FIBRINOID NECROSIS. Fibrinoid necrosis or fibrinoid degeneration is characterised by deposition of fibrin-like material which has the staining properties of fibrin. It is encountered in various examples of immunologic tissue injury (e.g. in immune complex vasculitis, autoimmune diseases, Arthus reaction etc), arterioles in hypertension, peptic ulcer etc.

Microscopically, fibrinoid necrosis is identified by brightly eosinophilic, hyaline-like deposition in the vessel wall or on the luminal surface of a peptic ulcer. Local haemorrhages may occur due to rupture of these blood vessels.

APOPTOSIS

Apoptosis is a form of 'coordinated and internally programmed cell death' which is of significance in a variety of physiologic and pathologic conditions (*apoptosis* is a Greek word meaning 'falling off' or 'dropping off'). The term was first coined in 1972 as distinct from necrosis.

PATHOLOGIC CHANGES. The characteristic morphologic changes in apoptosis as seen in histologic and electron microscopic examination are as under (Fig. 5.2,B):

1. Involvement of *single cells or small clusters of cells* in the background of viable cells. The apoptotic cells are round to oval shrunken masses of intensely eosinophilic cytoplasm containing condensed or fragmented nuclear chromatin. Characteristically, unlike necrosis, inflammatory response around apoptosis is absent.
2. *Shrinkage of cell* with dense cytoplasm and almost-normal organelles.
3. Convolutions of the cell membrane with formation of membrane-bound near-spherical bodies called *apoptotic bodies* containing compacted organelles.
4. *Chromatin condensation* around the periphery of nucleus.
5. Characteristically, there is *no acute inflammatory reaction*.
6. *Phagocytosis* of apoptotic bodies by macrophages takes place at varying speed. There may be swift phagocytosis, or loosely floating apoptotic cells after losing contact, with each other and basement membrane as single cells, or may result in major cell loss in the tissue without significant change in the overall tissue structure.

BIOCHEMICAL CHANGES. Biochemical processes underlying the morphologic changes are as under:

1. Proteolysis of cytoskeletal proteins.
2. Protein-protein cross linking.
3. Fragmentation of nuclear chromatin by activation of nuclease.
4. Appearance of phosphatidylserine on the outer surface of cell membrane.
5. In some forms of apoptosis, appearance of an adhesive glycoprotein thrombospondin on the outer surface of apoptotic bodies.
6. Appearance of phosphatidylserine and thrombospondin on the outer surface of apoptotic cell facilitates early recognition by macrophages for phagocytosis prior to appearance of inflammatory cells.

IDENTIFYING APOPTOTIC CELLS. Identifying and counting of apoptotic cells is possible by following methods:

1. Staining of chromatin condensation (by haematoxylin, Feulgen, or acridine orange).
2. Flow cytometry to visualise rapid cell shrinkage.
3. DNA changes detected by *in situ* techniques or by gel electrophoresis.
4. Annexin V as marker for apoptotic cell membrane having phosphatidylserine on the cell exterior.

MOLECULAR MECHANISMS OF APOPTOSIS.

Several physiologic and pathologic processes activate apoptosis in a variety of ways. However, in general the following events sum up the sequence involved in apoptosis:

1. Initiators of apoptosis. Stimuli for signalling programmed cell death act either at the cell membrane or intracellularly. These include:

- i) Absence of stimuli required for normal cell survival (e.g. absence of certain hormones, growth factors, cytokines).
- ii) Activators of programmed cell death (e.g. receptors for TNF).
- iii) Intracellular stimuli include heat, radiation, hypoxia etc.

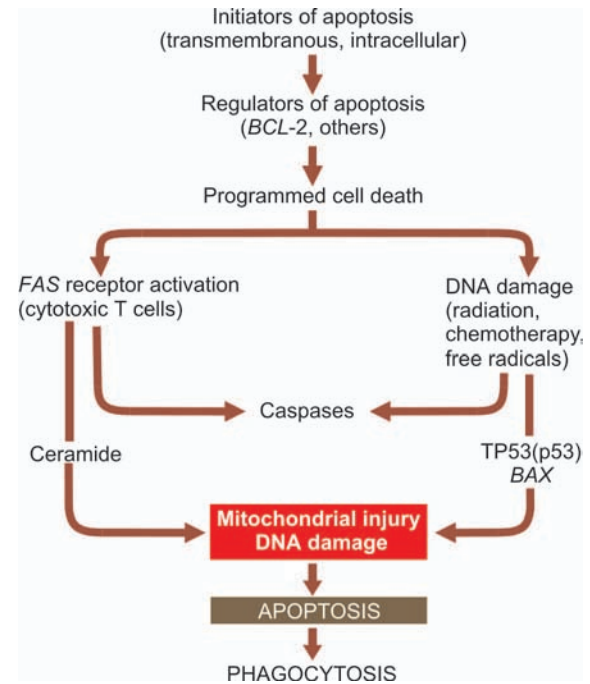
2. Regulators of apoptosis. After a cell has been initiated into apoptosis by above-mentioned signals, next comes the phase in which certain proteins convert death signals to the final programmed cell death and thus determine the outcome. These regulator proteins include the following:

- i) *BCL-2*. *BCL-2* protein is a human counterpart of *CED-9* (cell death) gene found in programmed cell death of nematode worm *C. elegans*. *BCL-2* is located in the outer mitochondrial membrane and may regulate the apoptotic process by binding to some other related proteins e.g to *BAX* and *BAD* for promoting apoptosis, and *BCL-XL* for inhibiting apoptosis. Another important *BCL-2* binding protein in the cytosol is the pro-apoptotic protease activating factor (*apaf-1*) which is a mammalian counterpart of gene *CED-4* of nematode. The net effect on the mitochondrial membrane is thus based on the pro-apoptotic and anti-apoptotic members of *BCL-2* protein family.
- ii) *Other apoptotic regulator proteins*. Besides *BCL-2*, other important regulator proteins of apoptosis are TP53 (*p53*) protein, *caspases*, *BAX* and certain viruses (adenovirus, papillomavirus, hepatitis B virus).

3. Programmed cell death. The final outcome of apoptotic regulators in the programmed cell death involves the following pathways:

i) *FAS receptor activation*. Cell surface receptor *FAS* (CD 95) is present on cytotoxic (CD 8+) T cells. On coming in contact with the target cell, the *FAS* receptor is activated. This leads to activation of caspases and subsequent proteolysis.

ii) *Ceramide generation*. Due to hydrolysis of phospholipid sphingomyelin of the plasma membrane, ceramide is generated. Ceramide is implicated in further mitochondrial injury.

**FIGURE 5.4**

Mechanism of apoptosis.

iii) *DNA damage*. Damage to DNA produced by various agents such as ionising radiation, chemotherapeutic agents, activated oxygen species lead to apoptosis. DNA damage affects nuclear protein TP53 (*p53*) which induces the synthesis of cell death promoting protein *BAX*.

4. Phagocytosis. The dead apoptotic cells and their fragments possess cell surface receptors which facilitate their identification by adjacent phagocytes. The phagocytosis is unaccompanied by any other inflammatory cells.

The mechanism of apoptosis is schematically represented in Fig. 5.4.

APOPTOSIS IN BIOLOGIC PROCESSES. Apoptosis is responsible for mediating cell death in a wide variety of physiologic and pathologic processes as under:

Physiologic Processes:

1. Organised cell destruction in sculpting of tissues during *development of embryo*.
2. Physiologic involution of cells in *hormone-dependent tissues* e.g. endometrial shedding, regression of lactating breast after withdrawal of breast feeding.
3. Normal cell destruction followed by *replacement proliferation* such as in intestinal epithelium.

TABLE 5.2: Contrasting Features of Apoptosis and Necrosis.

FEATURE	APOPTOSIS	NECROSIS
1. <i>Definition</i>	Programmed and coordinated cell death	Cell death along with degradation of tissue by hydrolytic enzymes
2. <i>Causative agents</i>	Physiologic and pathologic processes	Hypoxia, toxins
3. <i>Morphology</i>	i) No Inflammatory reaction ii) Death of single cells iii) Cell shrinkage iv) Cytoplasmic blebs on membrane v) Apoptotic bodies vi) Chromatin condensation vii) Phagocytosis of apoptotic bodies by macrophages	i) Inflammatory reaction always present ii) Death of many adjacent cells iii) Cell swelling initially iv) Membrane disruption v) Damaged organelles vi) Nuclear disruption vii) Phagocytosis of cell debris by macrophages
4. <i>Molecular changes</i>	i) Lysosomes and other organelles intact ii) Genetic activation by proto-oncogenes and oncosuppressor genes, and cytotoxic T cell-mediated target cell killing	i) Lysosomal breakdown with liberation of hydrolytic enzymes ii) Cell death by ATP depletion, membrane damage, free radical injury

4. *Involution of the thymus* in early age.

Pathologic Processes:

1. Cell death in tumours exposed to *chemotherapeutic agents*.
2. *Cell death by cytotoxic T cells* in immune mechanisms such as in graft-versus-host disease and rejection reactions.
3. *Cell death in viral infections* e.g. formation of Councilman bodies in viral hepatitis.
4. *Pathologic atrophy* of organs and tissues on withdrawal of stimuli e.g. prostatic atrophy after orchiectomy, atrophy of kidney or salivary gland on obstruction of ureter or ducts, respectively.
5. *Progressive depletion of CD4+T cells* in the pathogenesis of AIDS.
6. Cell death in response to *injurious agents* involved in causation of necrosis e.g. radiation, hypoxia and mild thermal injury.
7. In *degenerative diseases of CNS* e.g. in Alzheimer's disease, Parkinson's disease, and chronic infective dementias.

The contrasting features of apoptosis and necrosis are illustrated in Fig. 5.2 and summarised in Table 5.2.

GANGRENE

Gangrene is a form of necrosis of tissue with superadded putrefaction. The type of necrosis is usually coagulative due to ischaemia (e.g. in gangrene of the bowel, gangrene of limb). On the other hand, *gangrenous or necrotising inflammation* is characterised by primarily inflammation provoked by virulent bacteria resulting in massive tissue necrosis. Thus, the end-result of necrotising inflammation and gangrene is the same but

the way the two are produced, is different. The examples of necrotising inflammation are: gangrene lung, gangrene appendicitis, and noma (cancrum oris).

There are 3 main forms of gangrene—dry, wet and gas gangrene. In either type of gangrene, coagulative necrosis undergoes liquefaction by the action of putrefactive bacteria.

Dry Gangrene

This form of gangrene begins in the distal part of a limb due to ischaemia. The typical example is the dry gangrene in the toes and feet of an old patient due to arteriosclerosis. Other causes of dry gangrene foot include thromboangiitis obliterans (Buerger's disease), Raynaud's disease, trauma, ergot poisoning. It is usually initiated in one of the toes which is farthest from the blood supply, containing so little blood that even the invading bacteria find it hard to grow in the necrosed tissue. The gangrene spreads slowly upwards until it reaches a point where the blood supply is adequate to keep the tissue viable. A *line of separation* is formed at this point between the gangrenous part and the viable part.

PATHOLOGIC CHANGES. *Macroscopically*, the affected part is dry, shrunken and dark black, resembling the foot of a mummy. It is black due to liberation of haemoglobin from haemolysed red blood cells which is acted upon by hydrogen disulfide (H₂S) produced by bacteria resulting in formation of black iron sulfide. The line of separation usually brings about complete separation with eventual falling off of the gangrenous tissue if it is not removed surgically (Fig. 5.5).

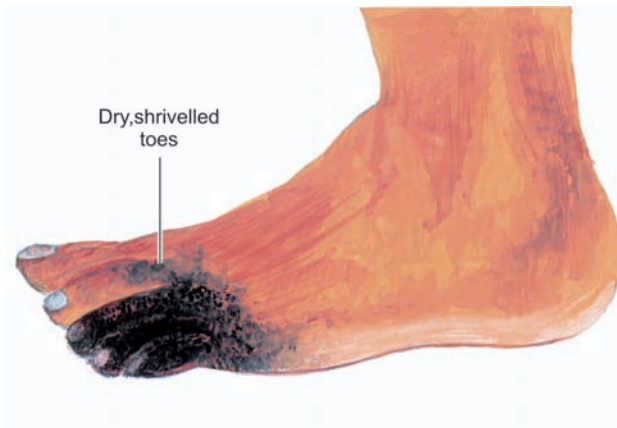


FIGURE 5.5

Dry gangrene of foot. The gangrenous area is dry, shrunk and dark and is separated from the viable tissue by clear line of separation.

Histologically, there is necrosis with smudging of the tissue. The line of separation consists of inflammatory granulation tissue.

Wet Gangrene

This occurs in naturally moist tissues and organs such as the mouth, bowel, lung, cervix, vulva etc. Diabetic foot is another example of wet gangrene due to high sugar content in the necrosed tissue which favours growth of bacteria. *Bedsore*s occurring in a bedridden

patient due to pressure on sites like the sacrum, buttocks and heels are the other important clinical conditions included in wet gangrene. Wet gangrene usually develops rapidly due to blockage of venous and less commonly arterial blood flow from thrombosis or embolism. The affected part is stuffed with blood which favours the rapid growth of putrefactive bacteria. The toxic products formed by bacteria are absorbed causing systemic manifestations of septicaemia, and finally death. The spreading wet gangrene lacks clear-cut line of demarcation and may spread to peritoneal cavity causing peritonitis.

PATHOLOGIC CHANGES. *Macroscopically*, the affected part is soft, swollen, putrid, rotten and dark. The classic example is gangrene of bowel, commonly due to strangulated hernia, volvulus or intussusception. The part is stained dark due to the same mechanism as in dry gangrene (Fig. 5.6,A).

Histologically, there is coagulative necrosis with stuffing of affected part with blood. There is ulceration of the mucosa and intense inflammatory infiltration. Lumen of the bowel contains mucus and blood. The line of demarcation between gangrenous segment and viable bowel is generally not clear-cut (Fig. 5.6,B) (COLOUR PLATE II: CL 5).

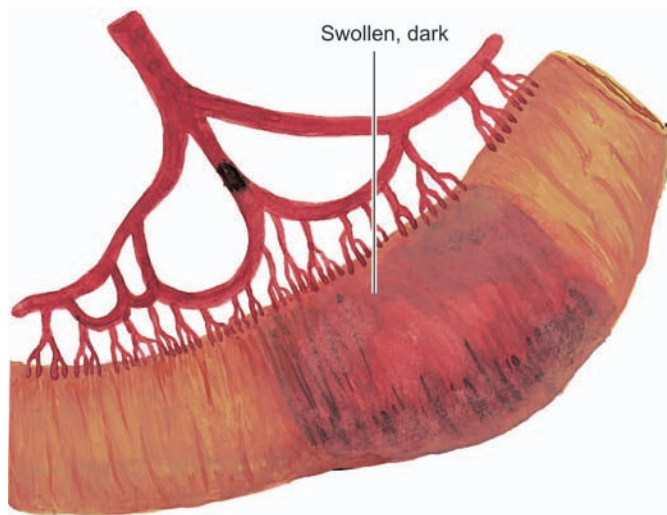


FIGURE 5.6

A, Wet gangrene of small bowel. The affected part is soft, swollen and dark. Line of demarcation between gangrenous segment and the viable bowel is not clear-cut. B, Microscopy shows coagulative necrosis of the affected bowel wall stuffed with blood while the junction with normal intestine shows an inflammatory infiltrate.

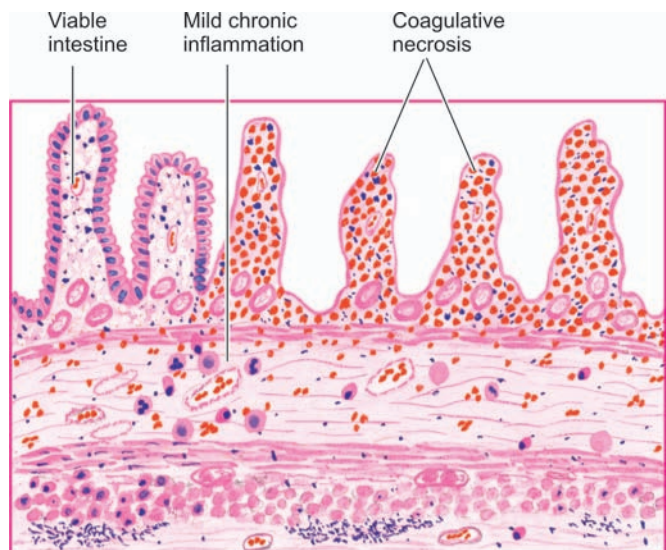


TABLE 5.3: Contrasting Features of Dry and Wet Gangrene.

FEATURE	DRY GANGRENE	WET GANGRENE
1. <i>Site</i>	Commonly limbs	More common in bowel
2. <i>Mechanisms</i>	Arterial occlusion	More commonly venous obstruction, less often arterial occlusion
3. <i>Macroscopy</i>	Organ dry, shrunken and black	Part moist, soft, swollen, rotten and dark
4. <i>Putrefaction</i>	Limited due to very little blood supply	Marked due to stuffing of organ with blood
5. <i>Line of demarcation</i>	Present at the junction between healthy and gangrenous part	No clear line of demarcation
6. <i>Bacteria</i>	Bacteria fail to survive	Numerous present
7. <i>Prognosis</i>	Generally better due to little septicaemia	Generally poor due to profound toxemia

Contrasting features of two main forms of gangrene are summarised in Table 5.3.

Gas Gangrene

Gas gangrene is a special form of wet gangrene caused by gas-forming clostridia (gram-positive anaerobic bacteria) which gain entry into the tissues through open contaminated wounds, especially in the muscles, or as a complication of operation on colon which normally contains clostridia. Clostridia produce various toxins which produce necrosis and oedema locally and are also absorbed producing profound systemic manifestations.

PATHOLOGIC CHANGES. *Grossly*, the affected area is swollen, oedematous, painful and crepitant due to accumulation of gas bubbles within the tissues. Subsequently, the affected tissue becomes dark black and foul smelling.

Microscopically, the muscle fibres undergo coagulative necrosis with liquefaction. Large number of gram-positive bacilli can be identified. At the periphery, a zone of leucocytic infiltration, oedema and congestion are found. Capillary and venous thrombi are common.

PATHOLOGIC CALCIFICATION

Deposition of calcium salts in tissues other than osteoid or enamel is called pathologic or heterotopic calcification. Two distinct types of pathologic calcification are recognised:

■ *Dystrophic calcification*, which is characterised by deposition of calcium salts in dead or degenerated tissues with normal calcium metabolism and normal serum calcium levels.

■ *Metastatic calcification*, on the other hand, occurs in apparently normal tissues and is associated with deranged calcium metabolism and hypercalcaemia.

Etiology and pathogenesis of the two are different but morphologically the deposits in both resemble normal minerals of the bone.

Histologically, in routine H and E stained sections, calcium salts appear as deeply basophilic, irregular and granular clumps. The deposits may be intracellular, extracellular, or at both locations. Occasionally, heterotopic bone formation (ossification) may occur. Calcium deposits can be confirmed by special stains like silver impregnation method of *von-Kossa* producing black colour, and *alizarin red S* that produces red staining. Pathologic calcification is often accompanied by diffuse or granular deposits of iron giving positive Prussian blue reaction.

Etiopathogenesis

The two types of pathologic calcification result from distinctly different etiologies and mechanisms.

DYSTROPHIC CALCIFICATION. As apparent from definition, dystrophic calcification may occur due to 2 types of causes:

- Calcification in dead tissue
- Calcification of degenerated tissue.

Calcification in dead tissue

1. *Caseous necrosis* in tuberculosis is the most common site for dystrophic calcification. Living bacilli may be present even in calcified tuberculous lesions, lymph nodes, lungs, etc.
2. *Liquefaction necrosis* in chronic abscesses may get calcified.

3. *Fat necrosis* following acute pancreatitis or traumatic fat necrosis in the breast results in deposition of calcium soaps.
4. *Infarcts* may sometimes undergo dystrophic calcification.
5. *Thrombi*, especially in the veins, may produce phleboliths.
6. *Haematomas* in the vicinity of bones may undergo dystrophic calcification.
7. *Dead parasites* like in hydatid cyst, *Schistosoma* eggs, and cysticercosis are some of the examples showing dystrophic calcification.
8. Calcification in *breast cancer* detected by mammo-graphy.
9. *Congenital toxoplasmosis* involving the central nervous system visualised by calcification in the infant brain.

Calcification in degenerated tissues

1. *Dense old scars* may undergo hyaline degeneration and subsequent calcification.
2. *Atheromas* in the aorta and coronaries frequently undergo calcification (COLOUR PLATE II: CL 6).
3. *Mönckeberg's sclerosis* shows calcification in the tunica media of muscular arteries in elderly people.
4. *Stroma of tumours* such as uterine fibroids, breast cancer, thyroid adenoma, goitre etc show calcification. Some tumours show characteristic spherules of calcification called *psammoma bodies* or calcospherites such as in meningioma, papillary serous cystadenocarcinoma of the ovary and papillary carcinoma of the thyroid.
5. *Cysts* which have been present for a long time may show calcification of their walls e.g. epidermal and pilar cysts.
6. *Calcinosis cutis* is a condition of unknown cause in which there are irregular nodular deposits of calcium salts in the skin and subcutaneous tissue.
7. *Senile degenerative changes* may be accompanied by dystrophic calcification such as in costal cartilages, tracheal or bronchial cartilages, and pineal gland in the brain etc.

The **pathogenesis** of dystrophic calcification is not quite clear. A few factors like local alteration of pH in the necrotic tissue and release of enzymes (e.g. alkaline phosphatase) from necrotic or degenerated tissue have been implicated which favour deposition of calcium salts. The process of dystrophic calcification has been likened to the formation of normal hydroxyapatite in the bone involving 2 phases: initiation and propagation.

■ *Initiation* is the phase in which calcium and phosphates begin to accumulate intracellularly in the mitochondria, or extracellularly in membrane-bound vesicles.

■ *Propagation* is the phase in which minerals deposited in the initiation phase are propagated to form mineral crystals.

METASTATIC CALCIFICATION. Since metastatic calcification occurs in normal tissues due to hypercalcaemia, its causes would include one of the following two conditions:

- Excessive mobilisation of calcium from the bone.
- Excessive absorption of calcium from the gut.

Excessive mobilisation of calcium from the bone. These causes are more common and include the following:

1. *Hyperparathyroidism* which may be primary such as due to parathyroid adenoma, or secondary such as from parathyroid hyperplasia, chronic renal failure etc.
2. *Bony destructive lesions* such as multiple myeloma, metastatic carcinoma.
3. *Prolonged immobilisation* of a patient results in disuse atrophy of the bones and hypercalcaemia.

Excessive absorption of calcium from the gut. Less often, excess calcium may be absorbed from the gut causing hypercalcaemia and metastatic calcification. These causes are as under:

1. *Hypervitaminosis D* results in increased calcium absorption.
2. *Milk-alkali syndrome* caused by excessive oral intake of calcium in the form of milk and administration of calcium carbonate in the treatment of peptic ulcer.
3. *Hypercalcaemia of infancy* is another condition in which metastatic calcification may occur.

Metastatic calcification may occur in any normal tissue of the body but affects the following organs more commonly:

- *Kidneys*, especially at the basement membrane of tubular epithelium and in the tubular lumina causing nephrocalcinosis.
- *Lungs*, especially in the alveolar walls.
- *Stomach*, on the acid-secreting fundal glands.
- *Blood vessels*, especially on the internal elastic lamina.
- *Cornea* is another site affected by metastatic calcification.

TABLE 5.4: Differences between Dystrophic and Metastatic Calcification.

FEATURE	DYSTROPHIC CALCIFICATION	METASTATIC CALCIFICATION
1. <i>Definition</i>	Deposits of calcium salts in dead and degenerated tissues	Deposits of calcium salts in normal tissues
2. <i>Calcium metabolism</i>	Normal	Deranged
3. <i>Serum calcium level</i>	Normal	Hypercalcaemia
4. <i>Causes</i>	Necrosis (caseous, liquefactive, fat), infarcts, thrombi, haematomas, dead parasites, old scars, atheromas, Mönckeberg's sclerosis, certain tumours, cysts, calcinosis cutis	Hyperparathyroidism (due to adenoma, hyperplasia, CRF), bony destructive lesions (e.g. myeloma, metastatic carcinoma), prolonged immobilisation, hypervitaminosis D, milk-alkali syndrome, hypercalcaemia of infancy
5. <i>Pathogenesis</i>	Akin to formation of normal hydroxyapatite involving phases of initiation and propagation	Favoured by relatively high pH (alkaline) at certain sites e.g. in lungs, stomach, blood vessels and cornea

The **pathogenesis** of metastatic calcification at the above mentioned sites is based on the hypothesis that these sites have relatively high pH (alkaline) which favours the precipitation of the calcium.

The distinguishing features between the two types of pathologic calcification are summarised in Table 5.4.



Intracellular accumulation of substances in abnormal amounts can occur within the cytoplasm (especially lysosomes) or nucleus of the cell. This phenomenon was previously referred to as *infiltration*, implying thereby that something unusual has infiltrated the cell from outside which is not always the case. Intracellular accumulation of the substance in mild degree causes reversible cell injury while more severe damage results in irreversible cell injury.

Such abnormal intracellular accumulations can be divided into 3 groups:

- i) *Accumulation of constituents of normal cell metabolism produced in excess* e.g. accumulations of lipids (fatty change, cholesterol deposits), proteins and carbohydrates. In addition, deposits of amyloid and urate are discussed later.
- ii) *Accumulation of abnormal substances* produced as a result of abnormal metabolism due to lack of some enzymes e.g. storage diseases or inborn errors of metabolism. These are discussed in Chapter 8.
- iii) *Accumulation of pigments* e.g. endogenous pigments under special circumstances, and exogenous pigments due to lack of enzymatic mechanisms to degrade the substances or transport them to other sites.

These pathologic states are discussed below.

FATTY CHANGE (STEATOSIS)

Fatty change, steatosis or fatty metamorphosis is the intracellular accumulation of neutral fat within parenchymal cells. It includes the older, now abandoned, terms of *fatty degeneration* and *fatty infiltration* because fatty change neither necessarily involves degeneration nor infiltration. The deposit is in the cytosol and represents an absolute increase in the intracellular lipids. It is especially common in the liver but may occur in other non-fatty tissues like the heart, skeletal muscle, kidneys (lipoid nephrosis or minimum change disease) and other organs.

Fatty Liver

Liver is the commonest site for accumulation of fat because it plays central role in fat metabolism. Depen-

ding upon the cause and amount of accumulation, fatty change may be mild and reversible, or severe producing irreversible cell injury and cell death.

ETIOLOGY. The commonest causes of fatty liver include the following:

- i) excess alcohol consumption (most common);
- ii) starvation;
- iii) malnutrition;
- iv) obesity;
- v) diabetes mellitus;
- vi) chronic illnesses (e.g. tuberculosis);
- vii) late pregnancy;
- viii) hypoxia (e.g. anaemia, cardiac failure);
- ix) hepatotoxins (e.g. carbon tetrachloride, chloroform, ether, aflatoxins and other poisons);
- x) certain drugs (e.g. administration of oestrogen, steroids, tetracycline etc); and
- xi) Reye's syndrome.

PATHOGENESIS. Pathogenesis of fatty liver depends upon the stage at which the individual etiologic agent acts in the normal fat transport and metabolism (Fig. 6.1).

Lipids as free acids enter the liver cell from either of the following 2 sources:

- *From diet* as chylomicrons (containing triglycerides and phospholipids) and as free fatty acids; and
- *From adipose tissue* as free fatty acids.

Normally, a small part of fatty acids is synthesised from acetate in the liver cells. Most of fatty acid is esterified to triglycerides by the action of α -glycerophosphate and only a small part is changed into cholesterol, phospholipids and ketone bodies. Intracellular triglycerides require 'lipid acceptor protein' to form lipoprotein, the form in which lipids are normally excreted from the liver cells.

In fatty liver, intracellular accumulation of triglycerides can occur due to defect at one or more of the following 6 steps in the normal fat metabolism:

1. Increased entry of free fatty acids into the liver.
2. Increased synthesis of fatty acids by the liver.
3. Decreased conversion of fatty acids into ketone bodies resulting in increased esterification of fatty acids to triglycerides.

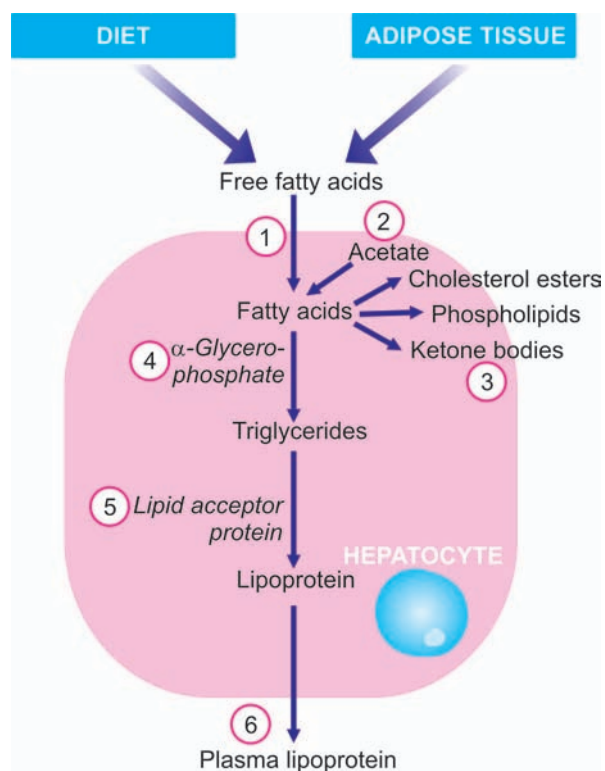


FIGURE 6.1

Lipid metabolism in the pathogenesis of fatty liver. Defects in any of the six numbered steps (corresponding to the description in the text) can produce fatty liver by different etiologic agents.

4. Increased α -glycerophosphate causing increased esterification of fatty acids to triglycerides.
5. Decreased synthesis of 'lipid acceptor protein' resulting in decreased formation of lipoprotein from triglycerides.
6. Block in the excretion of lipoprotein from the liver into plasma.

In most cases of fatty liver, one of the above mechanisms is operating. But in the case of liver cell injury by chronic alcoholism, many factors are implicated which includes:

- increased lipolysis;
- increased free fatty acid synthesis;
- decreased triglyceride utilisation;
- decreased fatty acid oxidation to ketone bodies; and
- block in lipoprotein excretion.

Even a severe form of liver cell dysfunction may be reversible; e.g. an alcoholic who has not developed progressive fibrosis in the form of cirrhosis, the enlarged fatty liver may return to normal if the person becomes teetotaler.

PATHOLOGIC CHANGES. *Grossly*, the fatty liver is enlarged with a tense, glistening capsule and rounded margins. The cut surface bulges slightly and is pale-yellow to yellow and is greasy to touch.

Microscopically, there are numerous lipid vacuoles in the cytoplasm of hepatocytes.

i) The vacuoles are initially small and are present around the nucleus (*microvesicular*).

ii) But with progression of the process, the vacuoles become larger pushing the nucleus to the periphery of the cells (*macrovesicular*) (**COLOUR PLATE I: CL 2**).

iii) At times, the hepatocytes laden with large lipid vacuoles may rupture and lipid vacuoles coalesce to form fatty cysts (Fig. 6.2).

iv) Infrequently, *lipogranulomas* may appear consisting of collections of lymphocytes, macrophages, and some multinucleated giant cells.

v) Fat in the tissue can be demonstrated by frozen section followed by *fat stains* such as Sudan dyes (Sudan III, IV, Sudan black), oil red O and osmic acid.

CHOLESTEROL. Intracellular deposits of cholesterol and its esters in macrophages may occur when there is hypercholesterolaemia. This turns macrophages into foam cells. The examples are:

1. *Fibrofatty plaques* of atherosclerosis (Chapter 24).

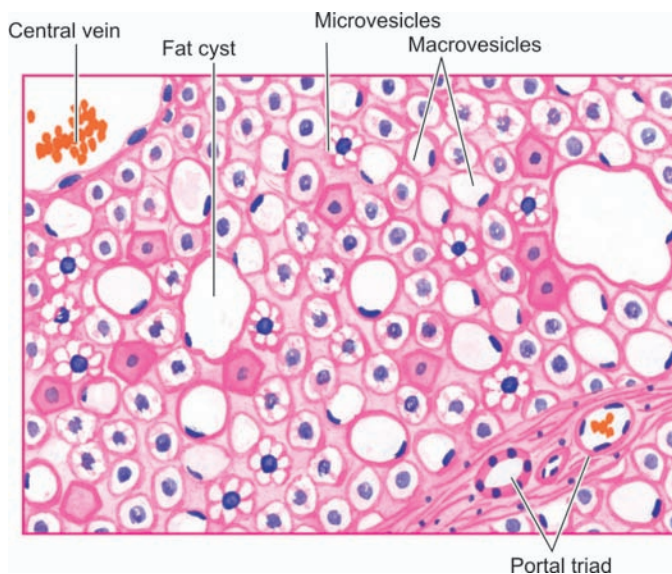


FIGURE 6.2

Fatty liver. Many of the hepatocytes are distended with fat vacuoles pushing the nuclei to the periphery, while others show multiple small vacuoles in the cytoplasm.

2. Clusters of foam cells in tumour-like masses called *xanthomas* and *xanthelasma*.

Stromal Fatty Infiltration

This form of lipid accumulation is quite different from fatty change just described. Stromal fatty infiltration is the deposition of mature adipose cells in the stromal connective tissue in contrast to intracellular deposition of fat in the parenchymal cells in fatty change. The condition occurs most often in patients with obesity. The two commonly affected organs are the heart and the pancreas. Thus, heart can be the site for intramyocardial fatty change as well as epicardial (stromal) fatty infiltration. The presence of mature adipose cells in the stroma generally does not produce any dysfunction. In the case of heart, stromal fatty infiltration is associated with increased adipose tissue in the epicardium.

INTRACELLULAR ACCUMULATION OF PROTEINS

Pathologic accumulation of proteins in the cytoplasm of cells may occur in the following conditions.

1. In *proteinuria*, there is excessive renal tubular re-absorption of proteins by the proximal tubular epithelial cells which show pink hyaline droplets in their cytoplasm. The change is reversible so that with control of proteinuria the protein droplets disappear.
2. The cytoplasm of actively functioning plasma cells shows pink hyaline inclusions called *Russell's bodies* representing synthesised immunoglobulins.
3. In α_1 -*antitrypsin deficiency*, the cytoplasm of hepatocytes shows eosinophilic globular deposits of a mutant protein.
4. *Mallory's body* or alcoholic hyaline in the hepatocytes is intracellular accumulation of intermediate filaments of cytokeratin and appear as amorphous pink masses.

INTRACELLULAR ACCUMULATION OF GLYCOGEN

Conditions associated with excessive accumulation of intracellular glycogen are as under:

1. In *diabetes mellitus*, there is intracellular accumulation of glycogen in different tissues because normal cellular uptake of glucose is impaired. Glycogen deposits in diabetes mellitus are seen in epithelium of distal portion of proximal convoluted tubule and descending loop of Henle, in the hepatocytes, in beta cells of pancreatic

islets, and in cardiac muscle cells. Deposits of glycogen produce clear vacuoles in the cytoplasm of the affected cells. Best's carmine and periodic acid-Schiff (PAS) staining may be employed to confirm the presence of glycogen in the cells.

2. In *glycogen storage diseases or glycogenosis*, there is defective metabolism of glycogen due to genetic disorders. These conditions along with other similar genetic disorders are discussed in Chapter 8.

PIGMENTS

Pigments are coloured substances present in most living beings including humans. There are 2 broad categories of pigments: endogenous and exogenous.

A. ENDOGENOUS PIGMENTS

Endogenous pigments are either normal constituents of cells or accumulate under special circumstances e.g. melanin, ochronosis, haemoprotein-derived pigments, and lipofuscin.

Melanin

Melanin is the brown-black, non-haemoglobin-derived pigment normally present in the hair, skin, choroid of the eye, meninges and adrenal medulla. It is synthesised in the melanocytes and dendritic cells, both of which are present in the basal cells of the epidermis and is stored in the form of cytoplasmic granules in the phagocytic cells called the melanophores, present in the underlying dermis. Melanocytes possess the enzyme tyrosinase necessary for synthesis of melanin from tyrosine. However, sometimes tyrosinase is present but is not active and hence no melanin pigment is visible. In such cases, the presence of tyrosinase can be detected by incubation of tissue section in the solution of dihydroxy phenyl alanine (DOPA). If the enzyme is present, dark pigment is identified in pigment cells. This test is called as *DOPA reaction* and is particularly useful in differentiating amelanotic melanoma from other anaplastic tumours.

Various disorders of melanin pigmentation cause generalised and localised hyperpigmentation and hypopigmentation:

i) Generalised hyperpigmentation:

- a) In *Addison's disease*, there is generalised hyperpigmentation of the skin, especially in areas exposed to light, and of buccal mucosa.
- b) *Chloasma* observed during pregnancy is the hyperpigmentation on the skin of face, nipples, and genitalia

and occurs under the influence of oestrogen. A similar appearance may be observed in women taking oral contraceptives.

c) In *chronic arsenical poisoning*, there is characteristic rain-drop pigmentation of the skin.

ii) Focal hyperpigmentation:

a) *Café-au-lait spots* are pigmented patches seen in neurofibromatosis and Albright's syndrome.

b) *Peutz-Jeghers syndrome* is characterised by focal perioral pigmentation.

c) *Melanos coli* is pigmentation of the mucosa of the colon.

d) *Melanotic tumours*, both benign such as pigmented naevi, and malignant such as melanoma, are associated with increased melanogenesis.

e) *Lentigo* is a pre-malignant condition in which there is focal hyperpigmentation on the skin of hands, face, neck, and arms.

f) *Dermatopathic lymphadenitis* is an example of deposition of melanin pigment in macrophages of the lymph nodes draining skin lesions.

iii) **Generalised hypopigmentation:** *Albinism* is an extreme degree of generalised hypopigmentation in which tyrosinase activity of the melanocytes is genetically defective and no melanin is formed. Albinos have blond hair, poor vision and severe photophobia. They are highly sensitive to sunlight. Chronic sun exposure may lead to precancerous lesions and squamous and basal cell cancers of the skin in such individuals.

iv) Localised hypopigmentation:

a) *Leucoderma* is a form of partial albinism and is an inherited disorder.

b) *Vitiligo* is local hypopigmentation of the skin and is more common. It may have familial tendency.

c) *Acquired focal hypopigmentation* can result from various causes such as leprosy, healing of wounds, DLE, radiation dermatitis etc.

Ochronosis

Ochronosis is an autosomal recessive disorder in which there is deficiency of an oxidase enzyme required for break down of homogentisic acid which then accumulates in the tissues and is excreted in the urine. The pigment is melanin-like and is deposited both intracellularly and intercellularly. Most commonly affected tissues are the cartilages, capsules of joints, ligaments and tendons. In almost all the cases, alkaptonuria is present in which homogentisic acid is excreted by the kidneys. The urine of these patients, if allowed to stand for some hours in air, turns black due to oxidation of homogentisic acid.

Haemoprotein-derived Pigments

Haemoproteins are the most important endogenous pigments derived from haemoglobin, cytochromes and their break down products. For an understanding of disorders of haemoproteins, it is essential to have knowledge of normal iron metabolism and its transport which is described in Chapter 34. In disordered iron metabolism and transport, haemoprotein-derived pigments accumulate in the body. These pigments are haemosiderin, acid haematin (haemozoin), bilirubin, and porphyrins.

1. **HAEMOSIDERIN.** Iron is stored in the tissues in 2 forms:

■ *Ferritin*, which is iron complexed to apoferritin and can be identified by electron microscopy.

■ *Haemosiderin*, which is formed by aggregates of ferritin and is identifiable by light microscopy as golden-yellow to brown, granular pigment, especially within the mononuclear phagocytes of the bone marrow, spleen and liver where break down of senescent red cells takes place. Haemosiderin is ferric iron that can be demonstrated by Prussian blue reaction. In this reaction, colourless potassium ferrocyanide reacts with ferric ions of haemosiderin to form deep blue *ferric-ferrocyanide* (COLOUR PLATE I: CL 4).

Excessive storage of haemosiderin occurs in situations when there is increased break down of red cells, or systemic overload of iron due to primary (idiopathic, hereditary) haemochromatosis, and secondary (acquired) causes such as in thalassaemia, sideroblastic anaemia, alcoholic cirrhosis, multiple blood transfusions etc.

Accordingly, the effects of haemosiderin excess are as under (Fig. 6.3):

a) **Localised haemosiderosis.** This develops whenever there is haemorrhage into the tissues. With lysis of red cells, haemoglobin is liberated which is taken up by macrophages where it is degraded and stored as haemosiderin. For example:

■ The changing colours of a bruise or a *black eye* are caused by the pigments like biliverdin and bilirubin which are formed during transformation of haemoglobin into haemosiderin.

■ Another example of local haemosiderosis is *brown induration* in the lungs as a result of small haemorrhages as occur in mitral stenosis and left ventricular failure. Microscopy reveals the presence of 'heart failure cells' which are haemosiderin-laden alveolar macrophages.

b) **Generalised (Systemic or Diffuse) haemosiderosis.** Systemic overload with iron may result in generalised haemosiderosis. There can be two types of patterns:

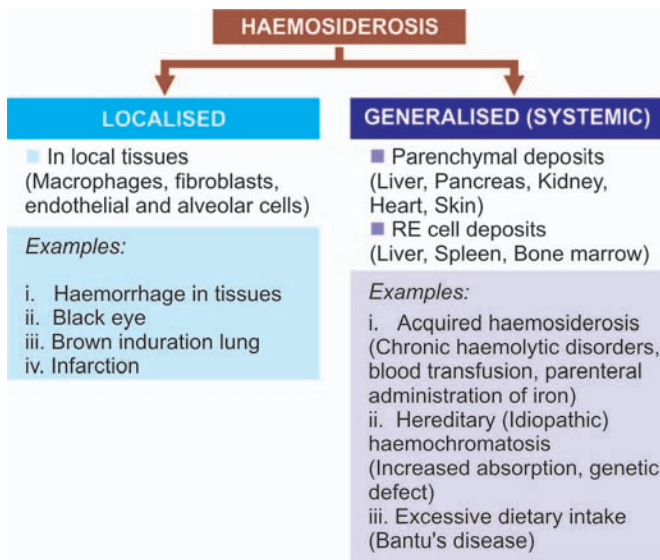


FIGURE 6.3

Effects of haemosiderosis.

■ *Parenchymatous deposition of haemosiderin* occurs in the parenchymal cells of the liver, pancreas, kidney, and heart.

■ *Reticuloendothelial deposition* occurs in the liver, spleen, and bone marrow.

Generalised or systemic overload of iron may occur due to following causes:

i) **Increased erythropoietic activity:** In various forms of chronic haemolytic anaemia, there is excessive break down of haemoglobin and hence iron overload. The problem is further compounded by treating the condition with blood transfusions (transfusional haemosiderosis) or by parenteral iron therapy. The deposits of iron in these cases, termed as *acquired haemosiderosis*, are initially in reticuloendothelial tissues but may secondarily affect other organs.

ii) **Excessive intestinal absorption of iron:** A form of haemosiderosis in which there is excessive intestinal absorption of iron even when the intake is normal, is known as *idiopathic or hereditary haemochromatosis*. It is an autosomal dominant disease associated with much more deposits of iron than cases of acquired haemosiderosis. It is characterised by *triad* of pigmentary liver cirrhosis, pancreatic damage resulting in diabetes mellitus, and skin pigmentation. On the basis of the last two features the disease has come to be termed as '*bronze diabetes*'.

iii) **Excessive intake of dietary iron:** A common example of excessive intake of iron is *Bantu's disease* in black

tribals of South Africa who conventionally brew their alcohol in cast iron pots that serves as a rich source of additional dietary iron. The excess of iron gets deposited in various organs including liver causing cirrhosis.

2. **ACID HAEMATIN (HAEMOZOIN).** Acid haematin or haemozoin is a haemoprotein-derived brown-black pigment containing haem iron in ferric form in acidic medium. But it differs from haemosiderin because it can not be stained by Prussian blue (Perl's) reaction, probably because of formation of complex with a protein so that it is unable to react in the stain. Haematin pigment is seen most commonly in chronic malaria and in mismatched blood transfusions. Besides, the *malarial pigment* can also be deposited in macrophages and in the hepatocytes. Another variety of haematin pigment is *formalin pigment* formed in blood-rich tissues which have been preserved in acidic formalin solution.

3. **BILIRUBIN.** Bilirubin is the normal non-iron containing pigment present in the bile. It is derived from porphyrin ring of the haem moiety of haemoglobin. Normal level of bilirubin in blood is less than 1 mg/dl. Excess of bilirubin causes an important clinical condition called jaundice. Normal bilirubin metabolism and pathogenesis of jaundice are described in Chapter 28. Briefly, jaundice appears in one of the following 3 ways:

- Prehepatic or haemolytic*, when there is excessive destruction of red cells.
- Posthepatic or obstructive*, which results from obstruction to the outflow of conjugated bilirubin.
- Hepatocellular* that results from failure of hepatocytes to conjugate bilirubin and inability of bilirubin to pass from the liver to intestine.

Excessive accumulation of bilirubin pigment can be seen in different tissues and fluids of the body, especially in the hepatocytes, Kupffer cells and bile sinusoids. Skin and sclerae become distinctly yellow. In infants, rise in unconjugated bilirubin may produce toxic brain injury called *kernicterus*.

4. **PORPHYRINS.** Porphyrins are tetrapyrroles which exist in 3 forms in nature combined with different metals:

- haem* contains iron;
- chlorophyll* contains magnesium; and
- cobalamin* contains cobalt.

Porphyria results from genetic deficiency of one of the enzymes required for the synthesis of haem so that there is excessive production of porphyrins. Often, the genetic deficiency is precipitated by intake of some drugs. Porphyrias are broadly of 2 types: erythropoietic and hepatic.

(a) **Erythropoietic porphyrias.** These have defective synthesis of haem in the erythrocytes. These may be further of 2 subtypes:

- *Congenital type*, in which the urine is red due to the presence of uroporphyrin I and coproporphyrin I. The skin of these infants is highly photosensitive. Bones and skin show red brown discolouration.

- *Erythropoietic protoporphyria*, in which there is excess of protoporphyrin but no excess of porphyrin in the urine.

(b) **Hepatic porphyrias.** These are more common and have a defect in synthesis of haem in the liver. Its further subtypes include the following:

- *Acute intermittent porphyria* is characterised by acute episodes of 3 patterns: abdominal, neurological, and psychotic. These patients do not have photosensitivity. There is excessive delta aminolaevulinic acid and porphobilinogen in the urine.

- *Variegate porphyria* is common in the whites of South Africa. Photosensitivity occurs and there are acute attacks of colicky abdominal pain and neurological manifestations.

- *Hereditary coproporphyria* is quite rare.

- *Porphyria cutanea tarda* is the most common of all porphyrias. Porphyrins collect in the liver and small quantity is excreted in the urine. Skin lesions are similar to those in variegate porphyria. Most of the patients have associated haemosiderosis with cirrhosis which may eventually develop into hepatocellular carcinoma.

Lipofuscin (Wear and Tear Pigment)

Lipofuscin or lipochrome is yellowish-brown intracellular lipid pigment (*lipo* = fat, *fuscus* = brown). The pigment is often found in atrophied cells of old age and hence the name 'wear and tear pigment'. It is seen in the myocardial fibres, hepatocytes, Leydig cells of the testes and in neurons in senile dementia. However, the pigment may, at times, accumulate rapidly in different cells in wasting diseases unrelated to aging.

By **light microscopy**, the pigment is coarse golden brown granular and often accumulates in the central part of the cells around the nuclei. In the heart muscle, the change is associated with wasting of the muscle and is commonly referred as 'brown atrophy' (Fig. 6.4). The pigment can be stained by fat stains but differs from other lipids in being fluorescent and acid-fast.

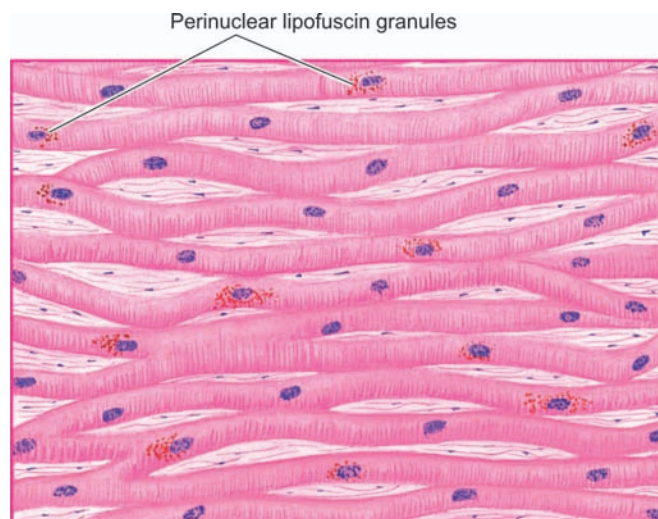


FIGURE 6.4

Brown atrophy of the heart. The lipofuscin pigment granules are seen in the cytoplasm of the myocardial fibres, especially at the poles of nuclei.

By electron microscopy, lipofuscin appears as intra-lysosomal electron-dense granules in perinuclear location. These granules are composed of lipid-protein complexes. Lipofuscin represents the collection of indigestible material in the lysosomes after intracellular lipid peroxidation and is therefore an example of residual bodies. Unlike in normal cells, in aging or debilitating diseases the phospholipid end products of membranes damage mediated by oxygen free radicals fail to get eliminated and hence are deposited as lipofuscin pigment.

B. EXOGENOUS PIGMENTS

Exogenous pigments are those which are introduced into the body from outside such as by inhalation, ingestion or inoculation.

Inhaled Pigments

The lungs of most individuals, especially of those living in urban areas due to atmospheric pollutants and of smokers, show a large number of inhaled pigmented materials. The most commonly inhaled substances are carbon or coal dust; others are silica or stone dust, iron or iron oxide, asbestos and various other organic substances. These substances may produce occupational lung diseases called pneumoconiosis. The pigment particles after inhalation are taken up by alveolar macrophages. Some of the pigment-laden macrophages

are coughed out via bronchi, while some settle in the interstitial tissue of the lung and in the respiratory bronchioles and pass into lymphatics to be deposited in the hilar lymph nodes. *Anthraxis* (i.e. deposition of carbon particles) is seen in almost every adult lung and generally provokes no reaction of tissue injury. However, extensive deposition of particulate material over many years in coal-miners' pneumoconiosis, silicosis, asbestosis etc. provoke low grade inflammation, fibrosis and impaired respiratory function.

Ingested Pigments

Chronic ingestion of certain metals may produce pigmentation. The examples are as under:

i) *Argyria* is chronic ingestion of silver compounds and results in brownish pigmentation in the skin, bowel, and kidney.

ii) *Chronic lead poisoning* may produce the characteristic blue lines on teeth at the gumline.

iii) *Melanos coli* results from prolonged ingestion of certain cathartics.

iv) *Carotenaemia* is yellowish-red colouration of the skin caused by excessive ingestion of carrots which contain carotene.

Injected Pigments (Tattooing)

Pigments like India ink, cinnabar and carbon are introduced into the dermis in the process of tattooing where the pigment is taken up by macrophages and lies permanently in the connective tissue. The examples of injected pigments are prolonged use of ointments containing mercury, dirt left accidentally in a wound, and tattooing by pricking the skin with dyes.



Amyloidosis

Amyloidosis is the term used for a group of diseases characterised by extracellular deposition of fibrillar proteinaceous substance called amyloid having common morphological appearance, staining properties and physical structure but with variable protein (or biochemical) composition.

First described by Rokitsky in 1842, the substance was subsequently named by Virchow as 'amyloid' under the mistaken belief that the material was starch-like (*amylon* = starch). This property was demonstrable *grossly* on the cut surface of an organ containing amyloid which stained brown with iodine and turned violet on addition of dilute sulfuric acid. By *light microscopy* with H&E staining, amyloid appears as extracellular, homogeneous, structureless and eosinophilic hyaline material, which is *positive with Congo red* staining and shows apple-green birefringence on *polarising microscopy*.

The nomenclature of different forms of amyloid is done by putting the alphabet A for amyloid, followed by the suffix derived from the name of specific protein constituting amyloid of that type e.g. AL (A for amyloid, L for light chain-derived), AA, ATTR etc.

PHYSICAL AND CHEMICAL NATURE OF AMYLOID

Ultrastructural examination and chemical analysis reveal the complex nature of amyloid. *It emerges that on the basis of morphology and physical characteristics, all forms of amyloid are similar in appearance, but they are chemically heterogeneous.* Based on these analysis, amyloid is composed of 2 main types of complex proteins (Fig. 7.1):

- I. *Fibril proteins* comprise about 95% of amyloid.
- II. *Non-fibrillar components* which include *P-component* predominantly and there are several different proteins which together constitute the remaining 5% of amyloid.

I. Fibril Proteins

By electron microscopy, it became apparent that major component of all forms of amyloid (about 95%) consists of meshwork of fibril proteins. The *fibrils* are delicate, randomly dispersed, non-branching, each measuring 7.5-10 nm in diameter and having indefinite length. Each fibril is further composed of double helix of two pleated sheets in the form of *twin filaments* separated by a clear

space. By X-ray crystallography and infra-red spectroscopy, the fibrils are shown to have *cross- β -pleated* sheet configuration which produces 1000 Å° periodicity that gives the characteristic staining properties of amyloid with Congo red and birefringence under polarising microscopy. Based on these features amyloid is also referred to as *β -fibrillosis*.

Chemical analysis of fibril proteins of amyloid revealed heterogeneous nature of amyloid. Chemically two major forms of amyloid fibril proteins were first identified in 1970s while currently 20 biochemically different proteins are known to form amyloid fibrils in humans in different clinicopathologic settings. Thus these can be categorised as under:

- i) AL (amyloid light chain) protein;
- ii) AA (amyloid associated) protein;
- iii) Other proteins.

AL PROTEIN. AL amyloid fibril protein is derived from immunoglobulin light chain, which in most cases includes amino-terminal segment of the immunoglobulin light chain and part of C region. AL fibril protein is more frequently derived from the lambda (λ) light chain than kappa (κ), the former being twice more common. However, in any given case, there is amino acid sequence homology. AL type of fibril protein is produced by immunoglobulin-secreting cells and is therefore seen in association with plasma cell dyscrasias and is seen in primary systemic amyloidosis.

AA PROTEIN. AA fibril protein is composed of protein with molecular weight of 8.5-kD which is derived from larger precursor protein in the serum called SAA (serum amyloid-associated protein) with a molecular weight of 12.5-kD. Unlike AL amyloid, the deposits of AA amyloid do not have sequence homology. In the plasma, SAA circulates in association with HDL₃ (high-density lipoprotein). SAA is an acute phase reactant protein synthesised in the liver, its level being high in chronic inflammatory and traumatic conditions. SAA fibril protein is found in secondary amyloidosis which includes the largest group of diseases associated with amyloidosis.

OTHER PROTEINS. Apart from the two major forms of amyloid fibril proteins, a few other forms of proteins are found in different clinical states:

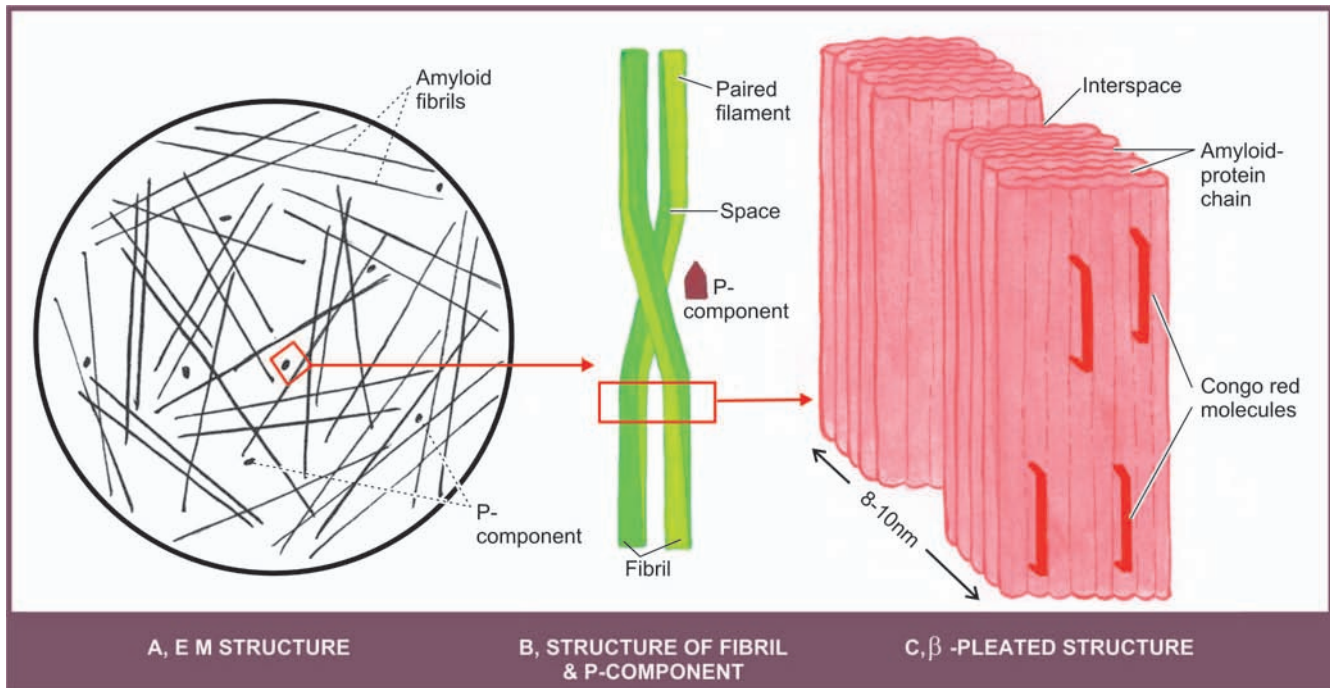


FIGURE 7.1

Diagrammatic representation of the ultrastructure of amyloid. A, Electron microscopy shows major part consisting of amyloid fibrils (95%) randomly oriented, while the minor part is essentially P-component (5%) B, Each fibril is further composed of double helix of two pleated sheets in the form of *twin filaments* separated by a clear space. P-component has a pentagonal or doughnut profile. C, X-ray crystallography and infra-red spectroscopy shows fibrils having *cross-β-pleated* sheet configuration which produces periodicity that gives the characteristic staining properties of amyloid with Congo red and birefringence under polarising microscopy.

1. Transthyretin (TTR) is a serum protein synthesised in the liver and transports *thyroxine* and *retinol* normally (trans-thy-retin). It was earlier called AFp (amyloid familial prealbumin) since it precedes albumin (pre-albumin) on serum electrophoresis but is not related to serum albumin. Single amino acid substitution mutations in the structure of TTR results in variant form of protein which is responsible for amyloidosis i.e. ATTR. About 60 such mutations have been described. ATTR is the most common form of heredofamilial amyloidosis e.g. in familial amyloid polyneuropathies. However, the deposits of ATTR in the elderly primarily involving the heart (senile cardiac amyloidosis) consists of normal TTR without any mutation. Another interesting aspect in ATTR is that despite being inherited, the disease appears in middle age or elderly.

2. Aβ₂-microglobulin (Aβ₂M) is amyloid seen in cases of long-term haemodialysis (8-10 years). As the name suggests, β₂M is a small protein which is a normal component of major histocompatibility complex (MHC) and has β-pleated sheet structure. β₂M is not effectively filtered during haemodialysis and thus there is high

serum concentration of β₂M protein in these patients. Although the deposit due to Aβ₂M may be systemic in distribution, it has predilection for bones and joints.

3. β-amyloid protein (Aβ) is distinct from Aβ₂M and is seen in cerebral plaques as well as cerebral blood vessels in Alzheimer's disease. Aβ is derived from amyloid beta precursor protein (AβPP) which is a transmembrane glycoprotein. The normal function of AβPP is probably cell-to-matrix signalling.

4. Immunoglobulin heavy chain amyloid (AH) derived from truncated heavy chain of immunoglobulin is an uncommon form of systemic amyloidosis.

5. Amyloid from hormone precursor proteins such as pro-calcitonin, islet amyloid polypeptide, pro-insulin, prolactin, atrial natriuretic factor, and lactoferrin has also been reported in amyloid.

6. Amyloid of prion protein (APrP) is derived from precursor prion protein which is a plasma membrane glycoprotein. Prion proteins are proteinaceous infectious particles lacking in RNA or DNA. Amyloid in prionosis occurs due to abnormally folded isoform of the PrP.

7. **Miscellaneous hereditary forms of amyloid** include a variety of amyloid proteins reported recently. These include amyloid derived from: apolipoprotein I (AApoAI), gelsolin (Agel), lysozyme (ALys), fibrinogen α -chain (AFib), and cystatin C (ACys) etc.

II. Non-fibrillar Components

Non-fibrillar components comprise about 5% of the amyloid material. These include the following:

1. **Amyloid P (AP)-component** synthesised in the liver and is present in all types of amyloid. It is derived from circulating serum amyloid P-component, a glycoprotein resembling the normal serum α_1 -glycoprotein and is PAS-positive. It is structurally related to C-reactive protein, an acute phase reactant, but is not similar to it. By electron microscopy, it has a pentagonal profile (P-component) or doughnut-shape with an external diameter of 9 nm and internal diameter of 4 nm.
2. **Apolipoprotein-E (apoE)**: It is a regulator of lipoprotein metabolism and is found in all types of amyloid. One allele apoE4 increases the risk of Alzheimer precursor protein (APP) deposition in Alzheimer's disease but not in all other types of amyloid deposits.
3. **Sulfated glycosaminoglycans (GAGs)**: These are constituents of matrix proteins; particularly associated is heparan sulfate in all types of tissue amyloid.
4. **α -1 anti-chymotrypsin**: It is only seen in cases of AA deposits but negative in primary amyloidosis.
5. **Protein X**: This protein has been shown to be present in cases of prionoses.
6. **Other components**: Besides above, components of complement, proteases, and membrane constituents may be seen.

PATHOGENESIS OF AMYLOIDOSIS

The earliest observation that amyloidosis developed in experimental animals who were injected repeatedly with antigen to raise antisera for human use led to the concept that amyloidogenesis was the result of immunologic mechanisms. AL variety of amyloid protein was thus first to be isolated. It is now appreciated that amyloidosis or fibrillogenesis is multifactorial and that different mechanisms are involved in different types of amyloid.

In general amyloidogenesis *in vivo* occurs in the following sequence (Fig. 7.2):

1. **Pool of amyloidogenic precursor protein** is present in circulation in different clinical settings and in response to stimuli e.g. increased hepatic synthesis of AA or ATTR, increased synthesis of AL etc.
2. **A nidus for fibrillogenesis**, meaning thereby an alteration in microenvironment to stimulate deposition of amyloid protein. This alteration involves changes and interaction between basement membrane proteins and amyloidogenic protein.

3. **Partial degradation or proteolysis** occurs prior to deposition of fibrillar protein which may occur in macrophages or reticuloendothelial cells e.g. in AL, AA, TTR, AGel, ACys, Alzheimer's disease, AApoAI.

4. **Exceptions to this generalisation** are seen in ATTR (hereditary type in which there are amino acid mutations in most cases), A β 2M (in which there are elevated levels of normal β 2M protein which remain unfiltered during haemodialysis) and prionosis (in which β -pleated sheet is formed *de novo*).

5. The role of **non-fibrillar components** such as AP, apoE and GAGs in amyloidosis is unclear; probably they facilitate in aggregation of proteins and protein folding leading to fibril formation, substrate adhesion and protection from degradation.

Deposition of AL and AA amyloid is schematically shown in Fig. 7.2 and is briefly outlined below.

Deposition of AL Amyloid

1. The stimulus for production of AL amyloid is some disorder of *immunoglobulin synthesis* e.g. multiple myeloma, B cell lymphoma, other plasma cell dyscrasias.
2. Excessive immunoglobulin production is in the form of *monoclonal gammopathy* i.e. there is production of either intact immunoglobulin, or λ light chain, or κ light chain, or rarely heavy chains. This takes place by monoclonal proliferation of plasma cells, B lymphocytes, or their precursors.
3. **Partial degradation** in the form of limited proteolysis of larger protein molecules occurs in macrophages which are anatomically closely associated with AL amyloid.
4. **Non-fibrillar components** like AP and GAGs play some role in folding and aggregation of fibril proteins.

Deposition of AA Amyloid

1. AA amyloid is directly related to **SAA levels**. SAA is a high density lipoprotein the levels of which are elevated in long-standing tissue destruction accompanied by chronic inflammation.
2. SAA is synthesised by the liver in response to *cytokines*, notably interleukin 1 and 6, from activated macrophages. However, SAA levels in isolation do not always lead to AA amyloid.
3. As in AL amyloid, **partial degradation** in the form of limited proteolysis takes place in reticuloendothelial cells.
4. In AA amyloid, a significant role is played by another glycoprotein, **amyloid enhancing factor (AEF)**. The exact composition of AEF is not known. It is elaborated in chronic inflammation, cancer and familial Mediterranean fever. On the basis of experimental induction of

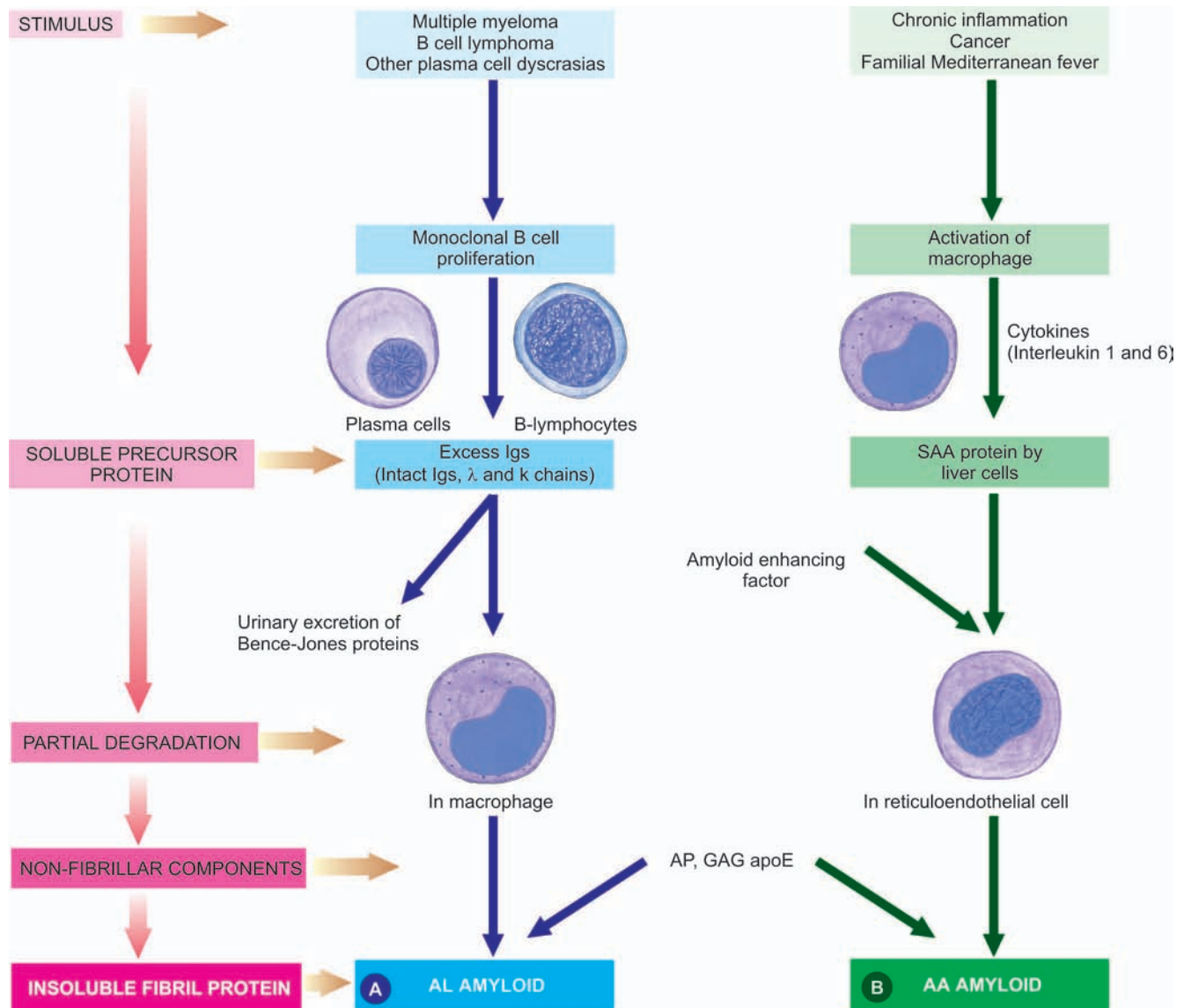


FIGURE 7.2

Pathogenesis of two main forms of amyloid deposition (AL = Amyloid light chain; AA = Amyloid-associated protein; GAG = glycosaminoglycan; AP = Amyloid P component). The sequence on left shows general schematic representation common to both major forms of amyloidogenesis.

AA amyloid, AEF has been shown to accelerate AA amyloid deposition. Possibly, AEF acts as a nidus for deposition of fibrils in AA amyloid.

5. As in AL amyloid, there is a role of *AP component* and *glycosaminoglycans* in the fibril protein aggregation and to protect it from disaggregation again.

CLASSIFICATION OF AMYLOIDOSIS

Amyloidosis has been classified in a number of ways in the past. These include:

- Classification of amyloidosis into *primary (mesenchymal)* and *secondary (parenchymal)* types;
- On histological basis into *pericollagenous* corresponding in distribution to primary, and *perireticulin* corresponding to secondary amyloidosis; and
- On clinical location of amyloidosis into *pattern I* (involving tongue, heart, bowel, skeletal and smooth muscle, skin and nerves), *pattern II* (principally involving liver, spleen, kidney and adrenals) and *mixed* (involving sites of both pattern I and II).

However, with availability of biochemical composition of various forms of amyloid, a biochemical-based *clinicopathologic classification* is widely acceptable (Table 7.1). According to this classification, amyloidosis can be divided into 2 major categories, each found in distinct clinical settings:

A. Systemic (generalised) amyloidosis:

1. Primary (AL)
2. Secondary/reactive/ inflammatory (AA)
3. Haemodialysis-associated ($A\beta_2M$)
4. Heredofamilial (ATTR, AA, Others)

B. Localised amyloidosis:

1. Senile cardiac (ATTR)
2. Senile cerebral ($A\beta$, APrP)
3. Endocrine (Hormone precursors)
4. Tumour-forming (AL)

A. SYSTEMIC AMYLOIDOSIS

1. Primary Systemic (AL) Amyloidosis

Primary amyloidosis consisting of AL fibril proteins is systemic or generalised in distribution. About 30% cases of AL amyloid have some form of plasma cell dyscrasias, most commonly multiple myeloma (in about 10% cases), and less often other monoclonal gammopathies such as

Waldenström's macroglobulinaemia, heavy chain disease, solitary plasmacytoma and nodular malignant lymphoma (B cell lymphoma). The neoplastic plasma cells usually are a single clone and, therefore, produce the same type of immunoglobulin light chain or part of light chain. Almost all cases of multiple myeloma have either λ or κ light chains (Bence Jones proteins) in the serum and are excreted in the urine. However, in contrast to normal or myeloma light chains, AL is twice more frequently derived from λ light chains.

The remaining 70% cases of AL amyloid do not have evident B-cell proliferative disorder or any other associated diseases and are thus cases of true 'primary' (idiopathic) amyloidosis. However, by more sensitive methods, some plasma cell dyscrasias are detectable in virtually all patients with AL. Majority of these cases too have a single type of abnormal immunoglobulin in their serum (monoclonal) and that these patients have some degree of plasmacytosis in the bone marrow, suggesting the origin of AL amyloid from precursor plasma cells.

AL amyloid is most prevalent type of systemic amyloidosis in the USA. Primary amyloidosis is often severe in the heart, kidney, bowel, skin, peripheral nerves, respiratory tract, skeletal muscle, and other organs.

TABLE 7.1: Classification of Amyloidosis.

CATEGORY	ASSOCIATED DISEASE	BIOCHEM. TYPE	ORGANS COMMONLY INVOLVED
A. SYSTEMIC (GENERALISED) AMYLOIDOSIS			
1. <i>Primary</i>	Plasma cell dyscrasias	AL type	Heart, bowel, skin, nerves, kidney
2. <i>Secondary (Reactive)</i>	Chronic inflammation, cancers	AA type	Liver, spleen, kidneys, adrenals
3. <i>Haemodialysis associated</i>	Chronic renal failure	$A\beta_2M$	Synovium, joints, tendon sheaths
4. <i>Heredofamilial</i>			
i. <i>Hereditary polyneuropathies</i>	—	ATTR	Peripheral and autonomic nerves, heart
ii. <i>Familial Mediterranean fever</i>	—	AA type	Liver, spleen, kidneys, adrenals
iii. <i>Rare hereditary forms</i>	---	AApoAI, AGel, ALys, AFib, ACys	Systemic amyloidosis
B. LOCALISED AMYLOIDOSIS			
1. <i>Senile cardiac</i>	Senility	ATTR	Heart
2. <i>Senile cerebral</i>	Alzheimer's, transmissible encephalopathy	$A\beta$, APrP	Cerebral vessels, plaques, neurofibrillary tangles
3. <i>Endocrine</i>	Medullary carcinoma Diabetes type II	Procalcitonin Proinsulin	Thyroid Islets of Langerhans
4. <i>Tumour-forming</i>	Lungs, larynx, skin, urinary bladder, tongue, eye	AL	Respective anatomic location

AL= Amyloid light chain; AA= Amyloid-associated protein; $A\beta_2M$ = Amyloid β_2 -microglobulin; ATTR= Amyloid transthyretin; APrP= Amyloid of prion proteins, $A\beta$ = β -amyloid protein).

Recently, it has been possible to reproduce AL amyloid in mice by repeated injections of human amyloidogenic light chains. Treatment of AL amyloid is targetted at reducing the underlying clonal expansion of plasma cells.

2. Secondary/Reactive (AA) Systemic Amyloidosis

The second form of systemic or generalised amyloidosis is reactive or inflammatory or secondary in which the fibril proteins contain AA amyloid. Secondary or reactive amyloidosis occurs typically as a complication of chronic infectious (e.g. tuberculosis, bronchiectasis, chronic osteomyelitis, chronic pyelonephritis, leprosy, chronic skin infections), non-infectious chronic inflammatory conditions associated with tissue destruction (e.g. autoimmune disorders such as rheumatoid arthritis, inflammatory bowel disease), some tumours (e.g. renal cell carcinoma, Hodgkin’s disease) and in familial Mediterranean fever, an inherited disorder (discussed below).

Secondary amyloidosis is typically distributed in solid abdominal viscera like the kidney, liver, spleen and adrenals. Secondary reactive amyloidosis is seen less frequently in developed countries due to containment of infections before they become chronic but this is the most common type of amyloidosis worldwide, particularly in underdeveloped and developing countries of the world.

AA amyloid occurs spontaneously in some birds and animals; it can also be experimentally induced in animals. Control of inflammation is the mainstay of treatment.

The contrasting features of the two main forms of systemic amyloidosis are given in Table 7.2.

3. Haemodialysis-Associated (Aβ₂M) Amyloidosis

Patients on long-term dialysis for more than 10 years for chronic renal failure may develop systemic amyloidosis derived from β₂-microglobulin which is normal component of MHC. The amyloid deposits are preferentially found in the vessel walls at the synovium, joints, tendon sheaths and subchondral bones. However, systemic distribution has also been observed in these cases showing bulky visceral deposits of amyloid.

4. Heredofamilial Amyloidosis

A few rare examples of genetically-determined amyloidosis having familial occurrence and seen in certain geographic regions have been described. These are as under:

- i) **Hereditary polyneuropathic (ATTR) amyloidosis.** This is an autosomal dominant disorder in which amyloid is deposited in the peripheral and autonomic nerves resulting in muscular weakness, pain and paraesthesia, or may have cardiomyopathy. This type of amyloid is derived from transthyretin (ATTR) with single amino acid substitution in the structure of TTR; about 60 types of such mutations have been described. Though hereditary, the condition appears well past middle life.
- ii) **Familial Mediterranean fever (AA).** This is an autosomal recessive disease and is seen in people of

TABLE 7.2: Contrasting Features of Primary and Secondary Amyloidosis.		
FEATURE	PRIMARY AMYLOID	SECONDARY AMYLOID
1. Biochemical composition	AL (Light chain proteins); lambda chains more common than kappa; sequence homology of chains	AA (Amyloid associated proteins); derived from larger precursor protein SAA; No sequence homology of polypeptide chain
2. Associated diseases	Plasma cell dyscrasias e.g. multiple myeloma, B cell lymphomas, others	Chronic inflammation e.g. infections (TB, leprosy, osteomyelitis, bronchiectasis), autoimmune diseases (rheumatoid arthritis, IBD), cancers (RCC, Hodgkin’s disease), FMF
3. Pathogenesis	Stimulus → Monoclonal B cell proliferation → Excess of Igs and light chains → Partial degradation → Insoluble AL fibril	Stimulus → Chronic inflammation → Activation of macrophages → Cytokines (IL1,6) → Partial degradation → AEF → Insoluble AA fibril
4. Incidence	Most common in US and other developed countries	Most common worldwide (particularly third world countries)
5. Organ distribution	Kidney, heart, bowel, nerves	Kidney, liver, spleen, adrenals
6. Stains to distinguish	Congophilia persists after permanganate treatment of section; specific immunostains anti-λ, anti-κ	Congophilia disappears after permanganate treatment of section; specific immunostain anti-AA

Mediterranean region (e.g. Sephardic jews, Armenians, Arabs and Turks). The condition is characterised by periodic attacks of fever and polyserositis i.e. inflammatory involvement of the pleura, peritoneum, and synovium causing pain in the chest, abdomen and joints respectively. Amyloidosis occurring in these cases is AA type, suggesting relationship to secondary amyloidosis due to chronic inflammation. The distribution of this form of heredofamilial amyloidosis is similar to that of secondary amyloidosis.

iii) Rare hereditary forms. Heredofamilial mutations of several normal proteins have been reported e.g. apolipoprotein I (AApoAI), gelsolin (Agel), lysozyme (ALys), fibrinogen α -chain (AFib), and cystatin C (ACys) etc. These types may also result in systemic amyloidosis.

B. LOCALISED AMYLOIDOSIS

1. Senile cardiac amyloidosis (ATTR). Senile cardiac amyloidosis is seen in 50% of people above the age of 70 years. The deposits are seen in the heart and aorta. The type of amyloid in these cases is ATTR but without any change in the protein structure of TTR.

2. Senile cerebral amyloidosis (A β , APrP). Senile cerebral amyloidosis is heterogeneous group of amyloid deposition of varying etiologies that includes sporadic, familial, hereditary and infectious. Some of the important diseases associated with cerebral amyloidosis and the corresponding amyloid proteins are: Alzheimer's disease (A β), Down's syndrome (A β) and transmissible spongiform encephalopathies (APrP) such as in Creutzfeldt-Jakob disease, fatal familial insomnia, mad cow disease, kuru.

In Alzheimer's disease, deposit of amyloid is seen as Congophilic angiopathy (amyloid material in the walls of cerebral blood vessels), neurofibrillary tangles and in senile plaques.

3. Endocrine amyloidosis (Hormone precursors). Some endocrine tumours are associated with microscopic deposits of amyloid e.g. in medullary carcinoma of the thyroid (procalcitonin), islet cell tumour of the pancreas (islet amyloid polypeptide), type 2 diabetes mellitus and insulinoma (pro-insulin), pituitary amyloid (prolactin), isolated atrial amyloid deposits (atrial natriuretic factor), and familial corneal amyloidosis (lactoferrin).

4. Localised tumour forming amyloid (AL). Sometimes, isolated tumour like formation of amyloid deposits are seen e.g. in lungs, larynx, skin, urinary bladder, tongue, eye, isolated atrial amyloid. In most of these cases, the amyloid type is AL.

STAINING CHARACTERISTICS OF AMYLOID

1. STAIN ON GROSS. The oldest method since the time of Virchow for demonstrating amyloid on cut surface of a gross specimen, or on the frozen/paraffin section is iodine stain. Lugol's iodine imparts *purple* colour to the amyloid-containing area which on addition of dilute sulfuric acid turns blue. This starch-like property of amyloid is due to AP component, a glycoprotein, present in all forms of amyloid.

Various stains and techniques employed to distinguish and confirm amyloid deposits in sections are as given in Table 7.3.

2. H & E. Amyloid by light microscopy with haematoxylin and eosin staining appears as extracellular, homogeneous, structureless and eosinophilic hyaline material, especially in relation to blood vessels. However, if the deposits are small, they are difficult to detect by routine H and E stains. Besides, a few other hyaline deposits may also take pink colour (page 34).

3. METACHROMATIC STAINS (ROSANILINE DYES). Amyloid has the property of metachromasia i.e. the dye reacts with amyloid and undergoes a colour change. Metachromatic stains employed are rosaniline dyes such as methyl violet and crystal violet which impart *rose-pink* colouration to amyloid deposits. However, small amounts of amyloid are missed, mucins also have metachromasia and that aqueous mountants are required for seeing the preparation. Therefore, this method has low sensitivity and lacks specificity.

4. CONGO RED AND POLARISED LIGHT. All types of amyloid have affinity for Congo red stain; therefore this method is used for confirmation of amyloid of all types. The stain may be used on both gross specimens and microscopic sections; amyloid of all types stains *red colour*. If the stained section is viewed in polarised

TABLE 7.3: Staining Characteristics of Amyloid.

STAIN	APPEARANCE
1. H & E	Pink, hyaline, homogeneous
2. Methyl violet/Crystal violet	Metachromasia: rose-pink
3. Congo red	Light microscopy: pink red Polarising light: red-green birefringence
4. Thioflavin-T/Thioflavin-S	Ultraviolet light: fluorescence
5. Immunohistochemistry (antibody against fibril protein)	Immunoreactivity: Positive
6. Non-specific stains:	
i) Standard toluidine blue	Orthochromatic blue, polarising ME dark red
ii) Alcian blue	Blue-green

light, the amyloid characteristically shows *apple-green birefringence* due to cross- β -pleated sheet configuration of amyloid fibrils. The stain can also be used to distinguish between AL and AA amyloid (primary and secondary amyloid respectively). After prior treatment with permanganate or trypsin on the section, Congo red stain is repeated—in the case of primary amyloid (AL amyloid), the Congo red positivity (congoophilia) persists,* while it turns negative for Congo red in secondary amyloid (AA amyloid). Congo red dye can also be used as an *in vivo* test (described below).

5. FLUORESCENT STAINS. Fluorescent stain thioflavin-T binds to amyloid and fluoresce *yellow* under ultraviolet light, i.e. amyloid emits secondary fluorescence. Thioflavin-S is less specific.

6. IMMUNOHISTOCHEMISTRY. More recently, type of amyloid can be classified by immunohistochemical stains. Various antibody stains against the specific antigenic protein types of amyloid are commercially available. However, more useful ones are *anti-AP* for confirmation of presence of amyloid of all types, and *anti-AA*, and *anti-lambda* (λ) and *anti-kappa* (κ) antibody stains for fibril protein of specific types by positive immunoreactivity.

7. NON-SPECIFIC STAINS. A few stains have been described for amyloid at different times but they lack specificity. These are as under:

i) Standard toluidine blue: This method gives *orthochromatic blue* colour to amyloid which under polarising microscopy produces *dark red* birefringence. However, there are false positive as well as false negative results; hence not recommended.

ii) Alcian blue: It imparts *blue-green* colour to amyloid positive areas and is used for mucopolysaccharide content in amyloid but uptake of dye is poor and variable.

iii) Periodic acid Schiff (PAS): It is used for demonstration of carbohydrate content of amyloid but shows variable positivity and is not specific.

DIAGNOSIS OF AMYLOIDOSIS

Amyloidosis may be detected as an unsuspected morphologic finding in a case, or the changes may be severe so as to produce symptoms and may even cause death. The diagnosis of amyloid disease can be made from the following investigations:

*Easy way to remember: Three *ps* i.e. persistence of congoophilia in primary amyloid after permanganate treatment.

1. BIOPSY EXAMINATION. Histologic examination of biopsy material is the commonest and confirmatory method for diagnosis in a suspected case of amyloidosis. Biopsy of an obviously *affected organ* is likely to offer the best results e.g. *kidney biopsy* in a case on dialysis, sural nerve biopsy in familial polyneuropathy. In systemic amyloidosis, renal biopsy provides the best detection rate, but *rectal biopsy* also has a good pick up rate. However, *gingiva* and *skin biopsy* have poor result. More recently, fine needle aspiration of abdominal *subcutaneous fat* followed by Congo red staining and polarising microscopic examination for confirmation has become an acceptable simple and useful technique with excellent result.

2. IN VIVO CONGO RED TEST. A known quantity of Congo red dye may be injected intravenously in living patient. If amyloidosis is present, the dye gets bound to amyloid deposits and its levels in blood rapidly decline. The test is, however, not popular due to the risk of anaphylaxis to the injected dye.

3. OTHER TESTS. A few other tests which are not diagnostic but are supportive of amyloid disease are protein electrophoresis, immunoelectrophoresis of urine and serum, and bone marrow aspiration.

PATHOLOGIC CHANGES IN AMYLOIDOSIS OF ORGANS

Although amyloidosis of different organs shows variation in morphologic pattern, some features are applicable in general to most of the involved organs.

Macroscopically, the affected organ is usually enlarged, pale and rubbery. Cut surface shows firm, waxy and translucent parenchyma which takes positive staining with the iodine test.

Microscopically, the deposits of amyloid are found in the extracellular locations, initially in the walls of small blood vessels producing microscopic changes and effects, while later the deposits are in large amounts causing macroscopic changes and effects of pressure atrophy.

Based on these general features of amyloidosis, the salient pathologic findings of major organ involvements are described below.

Amyloidosis of Kidneys

Amyloidosis of the kidneys is most common and most serious because of ill-effects on renal function. The deposits in the kidneys are found in most cases of secondary amyloidosis and in about one-third cases of

primary amyloidosis. Amyloidosis of the kidney accounts for about 20% of deaths from amyloidosis. Even small quantities of amyloid deposits in the glomeruli can cause proteinuria and nephrotic syndrome.

Grossly, the kidneys may be normal-sized, enlarged or terminally contracted due to ischaemic effect of narrowing of vascular lumina. Cut surface is pale, waxy and translucent.

Microscopically, amyloid deposition occurs primarily in the glomeruli, though it may involve peritubular interstitial tissue and the walls of arterioles as well (Fig. 7.3):

- *In the glomeruli*, the deposits initially appear on the basement membrane of the glomerular capillaries, but later extend to produce luminal narrowing and distortion of the glomerular capillary tuft. This results in abnormal increase in permeability of the glomerular capillaries to macromolecules with consequent proteinuria and nephrotic syndrome.

- *In the tubules*, the amyloid deposits likewise begin close to the tubular epithelial basement membrane. Subsequently, the deposits may extend further outwards into the intertubular connective tissue, and inwards to produce degenerative changes in the tubular epithelial cells and amyloid casts in the tubular lumina.

- The *vascular involvement* affects chiefly the walls of small arterioles and venules, producing narrowing of their lumina and consequent ischaemic effects (COLOUR PLATE I: CL 3)

Amyloidosis of Spleen

Amyloid deposition in the spleen, for some unknown reasons, may have one of the following two patterns (Fig. 7.4):

1. **'SAGO SPLEEN'**. The splenomegaly is not marked and cut surface shows characteristic translucent pale and waxy nodules resembling sago grains and hence the name.

Microscopically, the amyloid deposits begin in the walls of the arterioles of the white pulp and may subsequently replace the follicles.

2. **'LARDACEOUS SPLEEN'**. There is generally moderate to marked splenomegaly (weight up to 1 kg). Cut surface of the spleen shows map-like areas of amyloid (lardaceous-lard-like; *lard* means fat of pigs).

Microscopically, the deposits involve the walls of splenic sinuses and the small arteries and in the connective tissue of the red pulp.

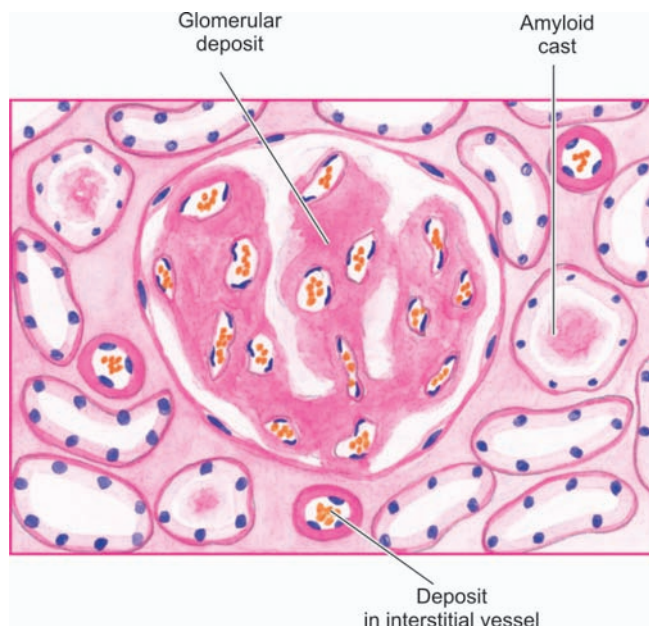


FIGURE 7.3

Amyloidosis of kidney. The amyloid deposits are seen mainly in the glomerular capillary tuft. The deposits are also present in peritubular connective tissue producing atrophic tubules and amyloid casts in the tubular lumina, and in the arterial wall producing luminal narrowing.

Amyloidosis of Liver

In about half the cases of systemic amyloidosis, liver involvement by amyloidosis is seen.

Grossly, the liver is often enlarged, pale, waxy and firm.

Microscopically, the features are as under (Fig. 7.5):

- The amyloid initially appears in the space of Disse (the space between the hepatocytes and sinusoidal endothelial cells).

- Later, as it increases, it compresses the cords of hepatocytes so that eventually the liver cells are shrunk and atrophic and replaced by amyloid. However, hepatic function remains normal even at an advanced stage of the disease.

- To a lesser extent, portal tracts and Kupffer cells are involved in amyloidosis.

Amyloidosis of Heart

Heart is involved in systemic amyloidosis quite commonly, more so in the primary than in secondary systemic amyloidosis. It may also be involved in localised form of amyloidosis in very old patients. Amyloidosis of the heart may produce arrhythmias due to deposition in the conduction system.

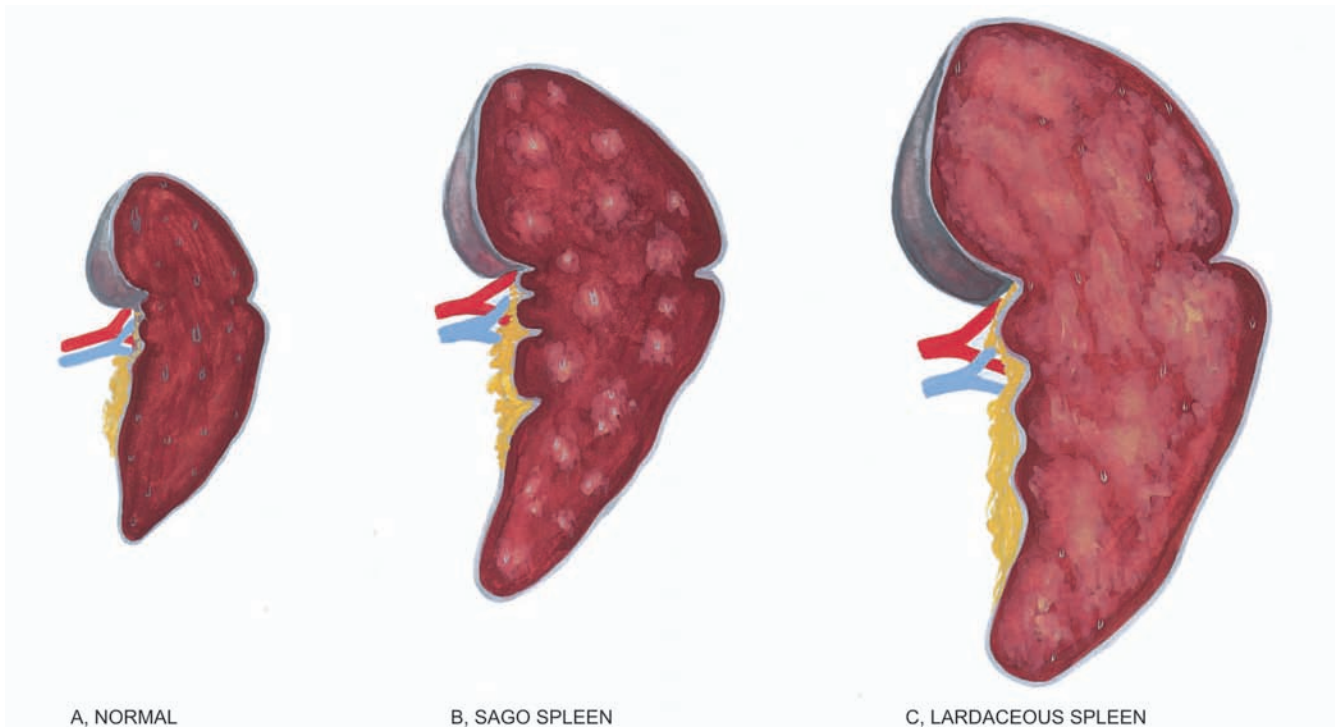


FIGURE 7.4

Macroscopic patterns of amyloidosis of the spleen.

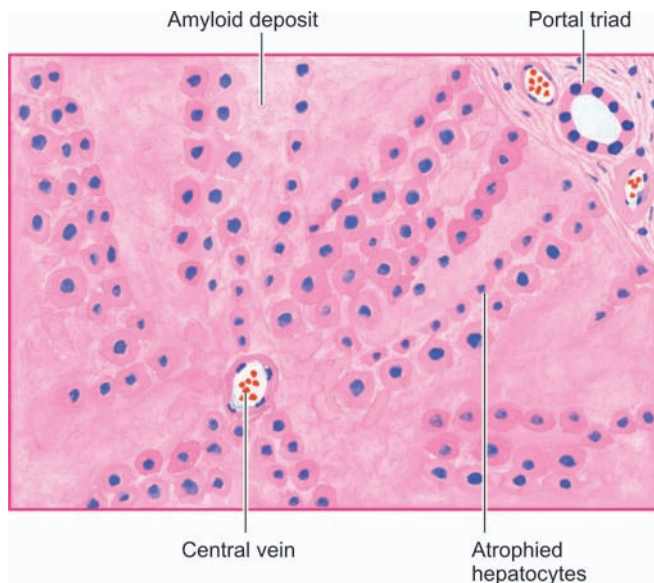


FIGURE 7.5

Amyloidosis of the liver. The deposition is extensive in the space of Disse causing compression and pressure atrophy of hepatocytes.

Grossly, the heart is often normal in size. However, at times it may be enlarged and show tiny nodular deposits of amyloid underneath the endocardium, subendocardially and between the myocardial fibres. Later, there may be a pressure atrophy of the myocardial fibres and impaired ventricular function which may produce restrictive cardiomyopathy.

Amyloidosis of Alimentary Tract

Involvement of the gastrointestinal tract by amyloidosis may occur at any level from the oral cavity to the anus. Rectal and gingival biopsies are the common sites for diagnosis of systemic amyloidosis. The deposits are initially located around the small blood vessels but later may involve adjacent layers of the bowel wall. Tongue may be the site for tumour-forming amyloid, producing macroglossia.

Other Organs

Uncommonly, the deposits of amyloid may occur in various other tissues such as pituitary, thyroid, adrenals,

skin, lymph nodes, respiratory tract and peripheral and autonomic nerves.

Thus, amyloidosis may be an incidental finding at autopsy or in symptomatic cases diagnosis can be made from the methods given above, biopsy examination being the most important method. The prognosis of

patients with generalised amyloidosis is generally poor; between the two forms of generalised amyloidosis, however, secondary reactive amyloidosis has somewhat better outcome due to controllable underlying condition. Renal failure and cardiac arrhythmias are the most common causes of death in systemic amyloidosis.



GENETIC DISEASES

This chapter deals with the group of disorders affecting the foetus during intrauterine life (developmental as well as genetic) and paediatric age group. In the western countries, developmental and genetic birth defects constitute about 50% of total mortality in infancy and childhood, while in the developing and underdeveloped countries 95% of infant mortality is attributed to environmental factors such as poor sanitation and undernutrition.

For the purpose of convenience of discussion, genetic and paediatric diseases are covered under the following headings:

1. *Developmental defects*: Errors in morphogenesis
2. *Cytogenetic (Karyotypic) defects*: Chromosomal abnormalities
3. *Single-gene defects*: Mendelian disorders
4. *Multifactorial inheritance disorders*
5. *Other paediatric diseases*

Broad overview of these disorders is presented below.

DEVELOPMENTAL DEFECTS

Developmental defects are a group of abnormalities during foetal life due to errors in morphogenesis. The branch of science dealing with the study of developmental anomalies is called *teratology*. Certain chemicals, drugs, physical and biologic agents are known to induce such birth defects and are called *teratogens*. The morphologic abnormality or defect of an organ or anatomic region of the body so produced is called *malformation*.

Pathogenesis

The teratogens may result in one of the following outcome:

- i) intrauterine death;
- ii) intrauterine growth retardation;
- iii) functional defects; and
- iv) malformation.

The **effects** of teratogens in inducing developmental defects are related to the following factors:

■ *Variable individual susceptibility to teratogen*: All patients exposed to the same teratogen do not develop birth defect.

■ *Intrauterine stage at which patient is exposed to teratogen*: Most teratogens induce birth defects during the first trimester of pregnancy.

■ *Dose of teratogen*: Higher the exposure dose of teratogen, greater the chances of inducing birth defects.

■ *Specificity of developmental defect for specific teratogen*: A particular teratogen acts in a particular way and induces the same specific developmental defect.

Classification

Various developmental anomalies resulting from teratogenic effects are categorised as under:

Agenesis means the complete absence of an organ e.g. unilateral or bilateral agenesis of kidney.

Aplasia is the absence of development of an organ with presence of rudiment or anlage e.g. aplasia of lung with rudimentary bronchus.

Hypoplasia is incomplete development of an organ not reaching the normal adult size e.g. microglossia.

Atresia refers to incomplete formation of lumen in hollow viscus e.g. oesophageal atresia.

Developmental dysplasia is defective development of cells and tissues resulting in abnormal or primitive histogenetic structures e.g. renal dysplasia (*Developmental dysplasia* is different from *dysplasia* in relation to precancerous lesions discussed on page 232).

Dystraphic anomalies are the defects resulting from failure of fusion e.g. spina bifida.

Ectopia or heterotopia refers to abnormal location of tissue at ectopic site e.g. pancreatic heterotopia in the wall of stomach.

Examples of Developmental Defects

Some common clinically important examples are given below:

1. Anencephaly-spina bifida complex. This is the group of anomalies resulting from failure to fuse

(dysraphy). While anencephaly results from failure of neural tube closure, spina bifida occurs from incomplete closure of the spinal cord and vertebral column, often in the lumbar region. The latter results in meningocele or myelomeningocele.

2. Thalidomide malformations. Thalidomide is the best known example of teratogenic drug used as a sedative by pregnant women in 1960s in England and Germany and resulted in high incidence of limb-reduction anomalies (phocomelia) in the newborns.

3. Foetal hydantoin syndrome. Babies born to mothers on anti-epileptic treatment with hydantoin have characteristic facial features and congenital heart defects.

4. Foetal alcohol syndrome. Ethanol is another potent teratogen. Consumption of alcohol by pregnant mother in first trimester increases the risk of miscarriages, still births, growth retardation and mental retardation in the newborn.

5. TORCH complex. Infection with TORCH group of organisms (*Toxoplasma*, *Rubella*, *Cytomegalovirus*, and *Herpes simplex*) during pregnancy is associated with multisystem anomalies and TORCH syndrome in the newborn (page 182).

6. Congenital syphilis. As discussed in Chapter 11, vertical transmission of syphilis from mother to foetus is characterised by Hutchinson's triad: interstitial keratitis, sensorineural deafness and deformed Hutchinson's teeth, along with saddle-nose deformity.

CYTOGENETIC (KARYOTYPIC) ABNORMALITIES

Human germ cells (ova and sperms) contain 23 chromosomes (haploid or N) while all the nucleated somatic cells of the human body contain 23 pairs of chromosomes (diploid or $2N$)—44 autosomes and 2 sex chromosomes, being XX in females (46, XX) and XY in males (46, XY). The branch of science dealing with the study of human chromosomal abnormalities is called cytogenetics (discussed in Chapter 2).

In a female, one of the two X chromosomes (paternal or maternal derived) is inactivated during embryogenesis as stated in *Lyon hypothesis*. This inactivation is passed to all the somatic cells while the germ cells in the female remain unaffected i.e. ovary will always have active X chromosome. Such an inactive X chromosome in the somatic cells in females lies condensed in the nucleus and is called as *sex chromatin* seen specifically in the somatic cells in females. *Nuclear sexing* can be done for genetic female testing by preparing and staining the smears of squamous cells scraped from oral cavity, or by identifying the *Barr body* in the circulating neutrophils as drumstick appendage attached to one of

the nuclear lobes (Fig. 8.1). A minimum of 30% cells positive for sex chromatin is indicative of genetically female composition.

Though chromosomes can be studied in any human nucleated cells, circulating lymphocytes are more often used for this purpose. The study is done by arresting the dividing cells in metaphase by colchicine and then spreading them on glass slide and staining them with Giemsa stain.

Karyotype is the photographic representation of the stained preparation of chromosomes.

Each chromosome is composed of a pair of identical double helix of chromosomal DNA called *chromatids*. The chromosomes are classified based on their length and location of the *centromere*; centromere is the point where the two chromatids cross each other (Fig. 8.2). The distal end of each chromosome is called telomere.

Based on centromeric location, they are classified into 3 groups:

■ *Metacentric chromosomes* (numbers 1, 3, 16, 19, 20) are those in which the centromere is exactly in the middle.

■ *Submetacentric chromosomes* (numbers 1, 3) in which the centromere divides the chromosomes into short arm (*p* arm; *petit* means short in French) and long arm (*q* arm; for letter next to *p*).

■ *Acrocentric chromosomes* (numbers 13, 14, 15, 21, 22, and Y) have very short arm and the centromere is eccentrically located.

Based on length of chromosomes, they are divided into 7 groups—A to G, called *Denver classification* adopted at a meeting in Denver, Colorado in US.

Chromosomal banding techniques are employed for study of classes of chromosomes. Chromosomal bands are unique alternate dark and light staining patterns. Banding techniques include:

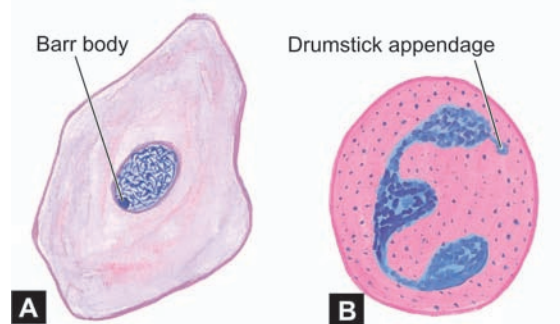


FIGURE 8.1

Nuclear sexing. A, sex chromatin as seen in scraped squamous cells from oral cavity. B, Barr body seen as drumstick appendage attached to a lobe of a circulating neutrophil.

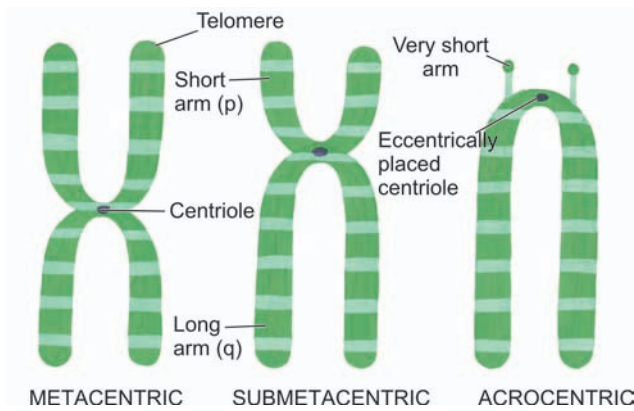


FIGURE 8.2

Classification of chromosomes based on size and location of centromere.

- i) G-banding (Giemsa stain);
- ii) Q-banding (quinacrine fluorescence stain);
- iii) R-banding (reverse Giemsa staining); and
- iv) C-banding (constitutive heterochromatin demonstration).

With these brief introductory comments, we can now turn to abnormalities of chromosomes which can be divided into 2 types:

1. Numerical abnormalities; and
2. Structural abnormalities.

Numerical Abnormalities

As mentioned above, normal karyotype of a human nucleated somatic cell is diploid or $2N$ (46 chromosomes) while the germ cells have haploid or $1N$ (23 chromosomes).

1. Polyploidy is the term used for the number of chromosomes which is a multiple of haploid number e.g. triploid or $3N$ (69 chromosomes), tetraploid or $4N$ (92 chromosomes). Polyploidy occurs normally in megakaryocytes and dividing liver cells. Polyploidy in somatic cells of conceptus results in spontaneous abortions.

2. Aneuploidy is the number of chromosomes which is not an exact multiple of haploid number e.g. hypodiploid or $2N-1$ (45 chromosomes) monosomy, hyperdiploid or $2N+1$ (47 chromosomes) trisomy.

The most common mechanism of aneuploidy is **nondisjunction**. Nondisjunction is the failure of chromosomes to separate normally during cell division during first or second stage of meiosis, or in mitosis.

■ *Nondisjunction during first meiotic division stage* will result in two gametes from both the parental chromosomes due to failure to separate while the other two gametes will have no chromosomes (nullisomic).

■ *Nondisjunction during second meiotic division stage* results in one gamete with two identical copies of the same chromosome, one nullisomic gamete, and two gametes with normal chromosome number.

■ *Nondisjunction during mitosis* results in mosaicism, meaning thereby that the individual has two or more types of cell lines derived from the same zygote. Mosaicism of mitotic nondisjunction of chromosomes occurs in cancers.

■ *Anaphase lag* is a form of nondisjunction involving single pair of chromosomes in which one chromosome in meiosis or a chromatid in mitosis fails to reach the pole of dividing cell at the same time (i.e. it lags behind) and is left out of the nucleus of daughter cell. This results in one normal daughter cell and the other monosomic for the missing chromosome.

Three clinically important syndromes resulting from numerical aberrations of chromosomes due to nondisjunction are as under and their main clinical features are illustrated in Fig. 8.3:

■ **Down's syndrome.** There is trisomy 21 in about 95% cases of Down's syndrome due to nondisjunction during meiosis in one of the parents. Down's syndrome is the most common chromosomal disorder and is the commonest cause of mental retardation. The incidence of producing offspring with Down's syndrome rises in mothers over 35 years of age.

■ **Klinefelter's syndrome.** Klinefelter's syndrome is the most important example of sex chromosome trisomy. About 80% cases have $47, XXY$ karyotype while others are mosaics. Typically, these patients have testicular dysgenesis. In general, sex chromosome trisomies are more common than trisomies of autosomes.

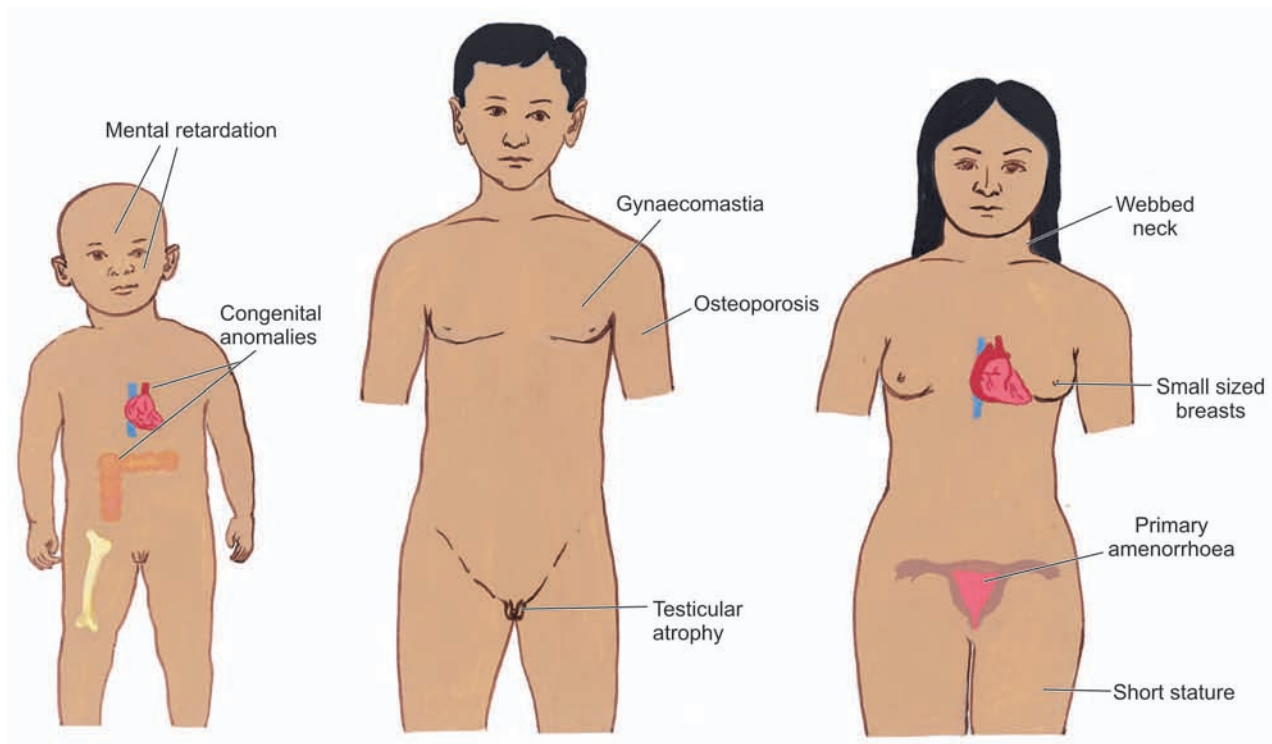
■ **Turner's syndrome.** Turner's syndrome is an example of monosomy ($45, X0$) most often due to loss of X chromosome in paternal meiosis.

Structural Abnormalities

During cell division (meiosis as well as mitosis), certain structural abnormalities of chromosomes may appear. These may occur during gametogenesis and then transmitted to all somatic cells and cause hereditary transmissible disorders, or may produce somatic cell mutations and result in changes varying from no effect to some forms of cancers. Structural abnormalities may be balanced or unbalanced.

■ *Balanced structural alteration* means no change in total number of genes or genetic material.

■ *Unbalanced structural alteration* refers to gene rearrangement resulting in loss or gain of genetic material.

**FIGURE 8.3**

Clinical features of important forms of numerical chromosomal abnormalities.

Some common forms of structural abnormalities are as under (Fig. 8.4):

TRANSLOCATIONS. Translocation means crossing over or exchange of fragment of chromosome which may occur between non-homologous or homologous chromosomes. There are two main types of translocations: reciprocal in about two-third and Robertsonian in one-third cases.

■ **Reciprocal translocation** is the exchange of genetic material between two non-homologous (heterologous) chromosomes without involving centromere (acentric). Such translocations occur due to single breaks in both the chromosomes and the exchange is detected by banding techniques. Reciprocal translocation may be balanced (without any loss of genetic material during the exchange) or unbalanced (with some loss of genetic material).

i) *Balanced reciprocal translocation* is more common and the individual is phenotypically normal e.g. translocation between long arm(q) of chromosomes 22 and long

arm(q) of chromosome 9 written as 46, XX, t (9;22). This translocation is termed Philadelphia chromosome seen in most cases of chronic myeloid leukaemia.

ii) *Unbalanced reciprocal translocations* are less common and account for repeated abortions and malformed children.

■ **Robertsonian translocation** is less common than reciprocal translocation. In this, there is fusion of two acrocentric chromosomes (having very short arms) at the centromere (centric fusion) with loss of short arms. The result of this fusion is one very large chromosome and the other very small one. Individuals born with Robertsonian translocation may be phenotypically normal but suffer from infertility and are at higher risk of producing malformed children in the next progeny.

DELETIONS. Loss of genetic material from the chromosome is called deletion. Deletion may be from the terminal or middle portion of the chromosome. The examples of deletion are: *cri du chat* (named after cry of infant like that of a cat) syndrome (deletion of short arm of chromosome 5) and several cancers with here-

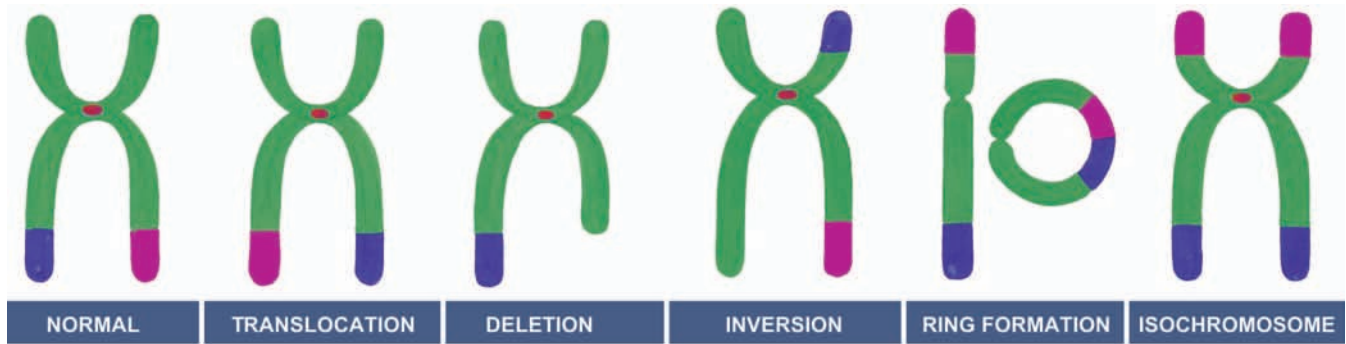


FIGURE 8.4

Common structural abnormalities of human chromosomes.

editary basis (e.g. retinoblastoma with deletion of long arm of chromosome 13, Wilms' tumour with deletion of short arm of chromosome 11).

INVERSION. Inversion is a form of rearrangement involving breaks of a single chromosome at two points. Inversion may be pericentric or paracentric, depending upon whether the rotation occurs at the centromere or at the acentric portion of the arm of chromosome. Inversions are not associated with any abnormality.

RING CHROMOSOME. A ring of chromosome is formed by a break at both the telomeric (terminal) ends of a chromosome followed by deletion of the broken fragment and then end-to-end fusion. The consequences of ring chromosome depend upon the amount of genetic material lost due to break.

ISOCHROMOSOME. When centromere rather than dividing parallel to the long axis, instead divides transverse to the long axis of chromosome, it results in either two short arms only or two long arms only called isochromosomes. The example involving isochromosome of X-chromosome is seen in some cases (15%) of Turner's syndrome.

SINGLE-GENE DEFECTS (MENDELIAN DISORDERS)

The classic laws of inheritance of characteristics or traits were outlined by Austrian monk Gregor Mendel in 1866 based on his observations of cross-breeding of red and white garden peas. Single-gene defects follow the classic mendelian patterns of inheritance and are also called mendelian disorders. These disorders are the result of mutation of a single gene of large effect.

MUTATIONS. The term mutation is applied to permanent change in the DNA of the cell. Mutations affecting germ cells are transmitted to the next progeny producing *inherited diseases*, while the mutations affecting somatic

cells give rise to *various cancers* and *congenital malformations*. Presently, following types of mutations have been described:

i) **Point mutation** is the result of substitution of a single nucleotide base by a different base i.e. replacement of an amino acid by another e.g. in sickle cell anaemia there is point mutation by substitution of glutamic acid by valine in the polypeptide chain.

ii) **Stop codon or nonsense mutation** refers to a type of point mutation in which the protein chain is prematurely terminated or truncated.

iii) **Frameshift mutation** occurs when there is insertion or deletion of one or two base pairs in the DNA sequence e.g. in cystic fibrosis of pancreas.

iv) **Trinucleotide repeat mutation** is characterised by amplification of a sequence of three nucleotides.

Thus it can be surmised from above that single-gene defects are synonymous with various types of heritable mutations. Currently, approximately 5000 single-gene defects have been described—some major and others of minor consequence. While most of these disorders are discussed in relevant chapters later, the group of storage diseases (inborn errors of metabolism) is considered below.

The inheritance pattern of genetic abnormalities may be *dominant or recessive, autosomal or sex-linked*. A *dominant gene** produces its effects, whether combined with similar dominant or recessive gene. *Recessive genes* are effective only if both genes are similar. However,

*A particular characteristic of an individual is determined by a pair of single *genes*, located at the same specific site termed *locus*, on a pair of homologous chromosomes. These paired genes are called *alleles* which may be *homozygous* when alike, and *heterozygous* if dissimilar. *Genotype* is the genetic composition of an individual while *phenotype* is the effect of genes produced.

when both alleles of a gene pair are expressed in heterozygote state, it is called *codominant inheritance*. A single gene may express in multiple allelic forms known as *polymorphism*. Genes on Y-chromosome are determinant for testis and are not known to cause any sex-linked disorder. Therefore, all sex-linked disorders are, in fact, X-linked disorders.

Table 8.1 lists important examples of groups of genetic disorders: *autosomal recessive* (the largest group), *codominant* (intermediate), and *dominant*, and *sex-(X-) linked recessive* and *dominant* disorders.

TABLE 8.1: Important Examples of Mendelian Disorders (Single-gene Defects).

I. AUTOSOMAL RECESSIVE INHERITANCE

1. β -thalassaemia
2. Sickle cell anaemia
3. Haemochromatosis
4. Cystic fibrosis of pancreas
5. Albinism
6. Wilson's disease
7. Xeroderma pigmentosum
8. Inborn errors of metabolism (Lysosomal storage diseases, glycogenosis, alkaptonuria, phenylketonuria)

II. AUTOSOMAL CODOMINANT INHERITANCE

1. ABO blood group antigens
2. α 1-antitrypsin deficiency
3. HLA antigens

III. AUTOSOMAL DOMINANT INHERITANCE

1. Familial polyposis coli
2. Adult polycystic kidney
3. Hereditary spherocytosis
4. Neurofibromatosis (von Recklinghausen's disease)
5. Marfan's syndrome
6. von Willebrand's disease
7. Hereditary haemorrhagic telangiectasia
8. Acute intermittent porphyria
9. Familial hypercholesterolaemia
10. Osteogenesis imperfecta

IV. SEX-(X-) LINKED RECESSIVE INHERITANCE

1. Haemophilia A
2. G6PD deficiency
3. Diabetes insipidus
4. Chronic granulomatous disease
5. Colour blindness
6. Bruton's agammaglobulinaemia
7. Muscular dystrophies

V. SEX-(X-) LINKED DOMINANT INHERITANCE

1. Hypophosphataemic rickets
2. Incontinentia pigmenti

STORAGE DISEASES (INBORN ERRORS OF METABOLISM)

Storage diseases or inborn errors of metabolism are biochemically distinct groups of disorders occurring due to genetic defect in the metabolism of carbohydrates, lipids, and proteins resulting in intracellular accumulation of metabolites. These substances may collect within the cells throughout the body but most commonly affected organ or site is the one where the stored material is normally found and degraded. Since lysosomes comprise the chief site of intracellular digestion (autophagy as well as heterophagy), the material is naturally stored in the lysosomes, and hence the generic name 'lysosomal storage diseases'. Cells of mononuclear-phagocyte system are particularly rich in lysosomes; therefore, reticuloendothelial organs containing numerous phagocytic cells like the liver and spleen are most commonly involved in storage disease.

Based on the biochemical composition of the accumulated material within the cells, storage diseases are classified into distinct groups, each group containing a number of diseases depending upon the specific enzyme deficiency. A summary of major groups of storage diseases alongwith their respective enzyme deficiencies, major accumulating metabolites and the organs involved is presented in Table 8.2. A few general comments can be made about all storage diseases:

■ All the storage diseases occur as a result of autosomal recessive, or sex-(X-) linked recessive genetic transmission.

■ Most, but not all, of the storage diseases are lysosomal storage diseases. Out of the glycogen storage diseases, only type II (Pompe's disease) is due to lysosomal enzyme deficiency.

A few important forms of storage diseases are described below:

Glycogen Storage Diseases (Glycogenoses)

These are a group of inherited disorders in which there is defective glucose metabolism resulting in excessive intracellular accumulation of glycogen in various tissues. Based on specific enzyme deficiencies, glycogen storage diseases are divided into 8 main types designated by Roman numerals I to VIII (Table 8.2). However, based on pathophysiology, glycogen storage diseases can be divided into 3 main subgroups:

1. Hepatic forms are characterised by inherited deficiency of hepatic enzymes required for synthesis of glycogen for storage (e.g. von Gierke's disease or type I glycogenosis) or due to lack of hepatic enzymes

TABLE 8.2: Storage Diseases (Inborn Errors of Metabolism).

DISEASE	ENZYME DEFICIENCY	ACCUMULATING METABOLITE	ORGANS INVOLVED
GLYCOGEN STORAGE DISEASE			
<i>Type I (von Gierke's disease)</i>	Glucose-6-phosphatase	Glycogen	Liver, kidney
<i>Type II (Pompe's disease)</i>	Acid- α -glucosidase (acid maltase)	Glycogen	Heart, skeletal muscle
<i>Type III (Forbes'/Cori's disease)</i>	Amylo-glucosidase (debrancher)	Limit dextrin	Heart, skeletal muscle
<i>Type IV (Anderson's disease)</i>	Amylo-trans-glucosidase (brancher)	Amylopectin	Liver
<i>Type V (McArdle's disease)</i>	Muscle phosphorylase	Glycogen	Skeletal muscle
<i>Type VI (Hers' disease)</i>	Liver phosphorylase	Glycogen	Liver
<i>Type VII</i>	Phosphofructokinase	Glycogen	Muscle
<i>Type VIII</i>	Phosphorylase kinase	Glycogen	Liver
MUCOPOLYSACCHARIDOSES (MPS)			
<i>Type I to type VI MPS syndromes</i>	Different lysosomal enzymes	Chondroitin sulphate, dermatan sulphate, heparan sulphate, keratan sulphate	Connective tissue, liver, spleen, bone marrow, lymph nodes, kidneys, heart, brain
SPHINGOLIPIDOSES (GANGLIOSIDOSES)			
<i>GM1-gangliosidosis (infantile and juvenile types)</i>	GM1 ganglioside-galactose	GM1-ganglioside	Liver, kidney, spleen, heart, brain
<i>GM2-gangliosidosis (Tay-Sachs, Sandhoff's disease)</i>	Hexosaminidase	GM2-ganglioside	Liver, kidney, spleen, heart, brain
SULFATIDOSES			
<i>Metachromatic leucodystrophy</i>	Aryl sulfatase A	Sulfatide	Brain, liver, spleen, heart, kidney
<i>Krabbe's disease</i>	Galactocerebrosidase	Galactocerebroside	Nervous system, kidney
<i>Fabry's disease</i>	α -Galactosidase	Ceramide	Skin, kidney, heart, spleen
<i>Gaucher's disease</i>	Glucocerebrosidase	Glucocerebroside	Spleen, liver, bone marrow
<i>Niemann-Pick disease</i>	Sphingomyelinase	Sphingomyelin	Spleen, liver, bone marrow, lymph nodes, lung

necessary for breakdown of glycogen into glucose (e.g. type VI glycogenosis).

2. Myopathic forms on the other hand, are those disorders in which there is genetic deficiency of glycolysis to form lactate in the striated muscle resulting in accumulation of glycogen in the muscles (e.g. McArdle's disease or type V glycogenosis, type VII disease).

3. Other forms are those in which glycogen storage does not occur by either hepatic or myopathic mechanisms. In Pompe's disease or type II glycogenosis, there is lysosomal storage of glycogen, while in type IV there is deposition of abnormal metabolites of glycogen in the brain, heart, liver and muscles.

The prototypes of these three forms are briefly considered below.

VON GIERKE'S DISEASE (TYPE I GLYCOGENOSIS). This condition is inherited as an autosomal recessive disorder due to deficiency of enzyme, glucose-6-phos-

phatase. In the absence of glucose-6-phosphatase, excess of normal type of glycogen accumulates in the liver and also results in hypoglycaemia due to reduced formation of free glucose from glycogen. As a result, fat is metabolised for energy requirement leading to hyperlipoproteinaemia and ketosis. Other changes due to deranged glucose metabolism are hyperuricaemia and accumulation of pyruvate and lactate.

The disease manifests clinically in infancy with failure to thrive and stunted growth. Most prominent feature is enormous hepatomegaly with intracytoplasmic and intranuclear glycogen. The kidneys are also enlarged and show intracytoplasmic glycogen in tubular epithelial cells. Other features include gout, skin xanthomas and bleeding tendencies due to platelet dysfunction.

POMPE'S DISEASE (TYPE II GLYCOGENOSIS). This is also an autosomal recessive disorder due to deficiency of a lysosomal enzyme, acid maltase, and is the only

example of *lysosomal* storage disease amongst the various types of glycogenoses. Acid maltase is normally present in most cell types and is responsible for the degradation of glycogen. Its deficiency, therefore, results in accumulation of glycogen in many tissues, most often in the heart and skeletal muscle, leading to cardiomegaly and hypotonia.

McARDLE'S DISEASE (TYPE V GLYCOGENOSIS).

The condition occurs due to deficiency of muscle phosphorylase resulting in accumulation of glycogen in the muscle (deficiency of liver phosphorylase results in type VI glycogenosis). The disease is common in 2nd to 4th decades of life and is characterised by painful muscle cramps, especially after exercise, and detection of myoglobinuria in half the cases.

Mucopolysaccharidoses (MPS)

Mucopolysaccharidoses are a group of six inherited syndromes numbered from MPS I to MPS VI. Each of these results from deficiency of specific lysosomal enzyme involved in the degradation of mucopolysaccharides or glycosaminoglycans, and are, therefore, a form of lysosomal storage diseases. Mucopolysaccharides which accumulate in the MPS are: chondroitin sulphate, dermatan sulphate, heparan sulphate and keratan sulphate. All these syndromes are autosomal recessive disorders except MPS II (Hunter's syndrome) which has X-linked recessive transmission.

Syndrome of MPS manifests in infancy or early childhood and involves multiple organs and tissues, chiefly connective tissues, liver, spleen, bone marrow, lymph nodes, kidneys, heart and brain. The mucopolysaccharides accumulate in mononuclear phagocytic cells, endothelial cells, intimal smooth muscle cells and fibroblasts. The material is finely granular and PAS-positive by light microscopy. By electron microscopy, it appears in the swollen lysosomes and can be identified biochemically as mucopolysaccharide.

Gaucher's Disease

This is an autosomal recessive disorder in which there is deficiency of lysosomal enzyme, glucocerebrosidase, which normally cleaves glucose from ceramide. This results in lysosomal accumulation of glucocerebroside (ceramide-glucose) in phagocytic cells of the body and sometimes in the neurons. The main sources of glucocerebroside in phagocytic cells are the membrane glycolipids of old leucocytes and erythrocytes, while the deposits in the neurons consist of gangliosides.

Clinically, 3 subtypes of Gaucher's disease are identified:

■ **Type I or classic form** is the adult form of disease in which there is storage of glucocerebrosides in the phagocytic cells of the body, principally involving the spleen, liver, bone marrow, and lymph nodes. This is the most common type comprising 80% of all cases of Gaucher's disease.

■ **Type II** is the infantile form in which there is progressive involvement of the central nervous system.

■ **Type III** is the juvenile form of the disease having features in between type I and type II i.e. they have systemic involvement like in type I and progressive involvement of the CNS as in type II.

The clinical features depend upon the clinical subtype of Gaucher's disease. In addition to involvement of different organs and systems (splenomegaly, hepatomegaly, lymphadenopathy, bone marrow and cerebral involvement), a few other features include pancytopenia, or thrombocytopenia secondary to hypersplenism, bone pains and pathological fractures.

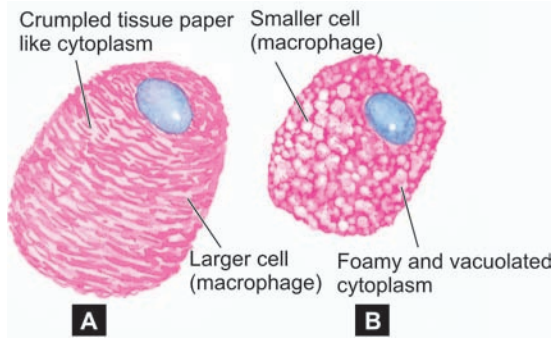
Microscopy shows large number of characteristically distended and enlarged macrophages called *Gaucher cells* which are found in the spleen, liver, bone marrow and lymph nodes, and in the case of neuronal involvement, in the Virchow-Robin space. The cytoplasm of these cells is abundant, granular and fibrillar resembling crumpled tissue paper. They have mostly a single nucleus but occasionally may have two or three nuclei (Fig. 8.5, A). Gaucher cells are positive with PAS, oil red O, and Prussian-blue reaction indicating the nature of accumulated material as glycolipids admixed with haemosiderin. These cells often show erythrophagocytosis and are rich in acid phosphatase.

Niemann-Pick Disease

This is also an autosomal recessive disorder characterised by accumulation of sphingomyelin and cholesterol. Majority of the cases (about 80%) have deficiency of sphingomyelinase which is required for cleavage of sphingomyelin, while a few cases probably result from deficiency of an activator protein.

The condition presents in infancy and is characterised by hepatosplenomegaly, lymphadenopathy and physical and mental underdevelopment. About a quarter of patients present with familial amaurotic idiocy with characteristic cherry-red spots in the macula of the retina (*amaurosis* = loss of vision without apparent lesion of the eye).

Microscopy shows storage of sphingomyelin and cholesterol within the lysosomes, particularly in the

**FIGURE 8.5**

A, Typical Gaucher cell. B, Typical macrophage in Niemann-Pick disease.

cells of mononuclear phagocyte system. The cells of Niemann-Pick disease are somewhat smaller than Gaucher cells and their cytoplasm is not wrinkled but is instead foamy and vacuolated which stains positively with fat stains (Fig. 8.5, B). These cells are widely distributed in the spleen, liver, lymph nodes, bone marrow, lungs, bowel and brain.

DISORDERS WITH MULTIFACTORIAL INHERITANCE

These are disorders which result from the combined effect of genetic composition and environmental influences. Some normal phenotypic characteristics have also multifactorial inheritance e.g. colour of hair, eye, skin, height and intelligence. Examples of disorders where environmental influences unmask the mutant genes are:

1. Cleft lip and cleft palate
2. Pyloric stenosis
3. Diabetes mellitus
4. Hypertension
5. Congenital heart disease
6. Coronary heart disease.

OTHER PAEDIATRIC DISEASES

As mentioned in the foregoing discussion, many diseases affecting infancy and childhood are genetic or developmental in origin. Here, we shall describe other diseases affecting the period from birth to puberty under the heading of paediatric diseases. This period is conventionally subdivided into 4 stages:

- *Neonatal period*: birth to first 4 weeks
- *Infancy*: first year of life
- *Early childhood*: 1-4 years
- *Late childhood*: 5-14 years

Each of these four stages has distinct anatomic, physiologic and immunologic development compared to adults and, therefore, has different groups of diseases unique to particular age groups. Before discussing these diseases affecting different age groups, a few general comments about these stages can be made:

1. Neonatal period is the period of continuation of dependent intrauterine foetal life to independent postnatal period. Therefore, this is the period of maximum risk to life due to perinatal causes (e.g. prematurity, low birth weight, perinatal infections, respiratory distress syndrome, birth asphyxia, birth trauma etc) and congenital anomalies. If adequate postnatal medical care is not provided, neonatal mortality is high. Neonatal mortality in first week after birth is about 10-times higher compared to second week, and shows improvement with every passing week at this stage.

2. In infancy, the major health problems are related to congenital anomalies, infections of lungs and bowel, and sudden infant death syndrome (often during sleep).

3. Young children from 1-4 years are exposed to higher risk of sustaining injuries, and manifest certain congenital anomalies. Some malignant tumours are peculiar to this age group.

4. Older children from 5-14 years too have higher risk of injuries from accidents and have other problems related to congenital anomalies and certain malignant tumours at this age.

Thus, hazardous effects of congenital anomalies are a common denominator for all age groups from birth to adolescence and have been discussed already. Tumours peculiar to infants and children are discussed along with discussion in related chapters of Systemic Pathology. However, a short note on general aspects of this subject is given below.

TUMOURS OF INFANCY AND CHILDHOOD

Tumours of infancy and childhood comprise 2% of all malignant tumours but they are the leading cause of death in this age group exceeded only by accidents. Benign tumours are more common than malignant neoplasms but they are generally of little immediate consequence. Another aspect requiring consideration here is the difficulty in differentiating benign tumours from tumour-like lesions discussed below.

HISTOGENESIS. Histogenetic evolution of tumours at different age groups takes place as under:

- Some tumours have probably evolved *in utero* and are apparent at birth or in immediate postnatal period. Such tumours are termed *developmental tumours*.

■ Many other tumours originate in abnormally developed organs and organ rests; they become apparent subsequently and are termed *embryonic tumours*.

■ In embryonic tumours, proliferation of embryonic cells occurs which *have not reached the differentiation stage* essential for specialised functions i.e. the cells proliferate as *undifferentiated or as partially differentiated* stem cells and an embryonal tumour is formed.

■ Tumours of infancy and childhood have *some features of normal embryonic or foetal cells* in them which proliferate under growth promoting influence of oncogenes and suffer from mutations which make them appear morphologically malignant.

■ Under appropriate conditions, these malignant embryonal cells may *cease to proliferate* and transform into non-proliferating mature differentiated cells e.g. a neonatal neuroblastoma may mature and differentiate into benign ganglioneuroma; foetal sacrococcygeal teratoma matures with age to adult tissues and is assigned better prognosis.

Thus, normal somatic cell maturation and neoplastic development in embryonal tumours represent two opposite ends of *ontogenesis*, with capability of some such tumours to mature and differentiate to turn benign from malignant.

A list of common benign tumours and tumour-like lesions is presented in Table 8.3.

Benign Tumours and Tumour-like Conditions

Many of the benign tumours seen in infancy and childhood are actually growth of displaced cells and masses of tissues and their proliferation takes place alongwith

the growth of the child. Some of these tumours undergo a phase of spontaneous regression subsequently—a feature usually not seen in true benign tumours. While some consider such lesions as mere '*tumour-like lesions or malformations*', others call them benign tumours. A few such examples are as under:

1. Hamartomas. Hamartomas are focal accumulations of cells normally present in that tissue but are arranged in an abnormal manner i.e. though present at normal site they do not reproduce normal architecture identical to adjacent tissues (page 236).

2. Choristoma (heterotopia). Choristoma or heterotopia is collection of normal cells and tissues at aberrant locations e.g. heterotopic pancreatic tissue in the wall of small bowel or stomach.

Malignant Tumours

Cancers of infancy and childhood differ from those in adults in the following respects:

1. Sites. Cancers of this age group more commonly pertain to haematopoietic system, neural tissue and soft tissues compared to malignant tumours in adults at sites such as the lung, breast, prostate, colon and skin.

2. Genetic basis. Many of paediatric malignant tumours have underlying genetic abnormalities.

3. Regression. Foetal and neonatal malignancies have a tendency to regress spontaneously or to mature.

4. Histologic feature. These tumours have unique histologic feature in having primitive or embryonal appearance rather than pleomorphic-anaplastic histologic appearance.

TABLE 8.3: Common Paediatric Benign Tumours and Tumour-like Lesions.

NOMENCLATURE	MAIN FEATURES
BENIGN TUMOURS	
i. <i>Haemangioma</i>	• Most common in infancy • Commonly on skin (e.g. port-wine stain) • May regress spontaneously
ii. <i>Lymphangioma</i>	• Cystic and cavernous type common • Located in skin or deeper tissues • Tends to increase in size after birth
iii. <i>Sacrococcygeal teratoma</i>	• Often accompanied with other congenital malformations • Majority (75%) are benign; rest are immature or malignant
iv. <i>Fibromatosis</i>	• Solitary (which generally behaves as benign) to multifocal (aggressive lesions)
TUMOUR-LIKE LESIONS/BENIGN TUMOURS	
i. <i>Naevocellular naevi</i>	• Very common lesion on the skin
ii. <i>Liver cell adenoma</i>	• Most common benign tumour of liver
iii. <i>Rhabdomyoma</i>	• Rare foetal and cardiac tumour

TABLE 8.4: Common Paediatric Malignant Tumours.

SYSTEM	AGE < 4 YRS	AGE 5-9 YRS	AGE 10-14 YRS
1. <i>Haematopoietic</i>	Acute leukaemia —	Acute leukaemia Lymphoma	— Hodgkin's
2. <i>Blastomas</i>	Neuroblastoma Hepatoblastoma Retinoblastoma Nephroblastoma (Wilms' tumour)	Neuroblastoma Hepatocellular carcinoma	— Hepatocellular carcinoma
3. <i>Soft tissues</i>	Rhabdomyosarcoma	Soft tissue sarcoma	Soft tissue sarcoma
4. <i>Bony</i>	—	Ewing's sarcoma	Osteogenic sarcoma
5. <i>Neural</i>	CNS tumours	CNS tumours	—
6. <i>Others</i>	Teratoma	—	Thyroid cancer

5. Management. Many of paediatric malignant tumours are curable by chemotherapy and/or radiotherapy but may develop second malignancy.

A few *generalisations* can be drawn about these cancers:

- In infants and children under 4 years of age: the most common malignant tumours are various types of *blastomas*.

- Children between 5 to 9 years of age: *haematopoietic malignancies* are more common.

- In the age range of 10-14 years (prepubertal age): *soft tissue and bony sarcomas* are the prominent tumours.

Based on these broad guidelines, classification of common paediatric malignant tumours at different age groups is presented in Table 8.4.



Environmental and Nutritional Diseases

ENVIRONMENTAL DISEASES

The subject of environmental hazards to health has assumed great significance in the modern world. In olden times, the discipline of 'tropical medicine' was of interest to the physician, largely due to contamination of air, food and water by infectious and parasitic organisms. Subsequently, the interest was focussed on 'geographic pathology' due to occurrence of certain environment-related diseases confined to geographic boundaries. Then emerged the knowledge of 'occupational diseases' caused by overexposure to a pollutant by virtue of an individual's occupation. Currently, the field of 'environmental pathology' encompasses all such diseases caused by progressive deterioration in the environment, most of which is man-made. In addition, is the related problem of over- and undernutrition.

Some of the important factors which have led to the alarming environmental degradation are as under:

1. Population explosion
2. Urbanisation of rural and forest land to accommodate the increasing numbers
3. Accumulation of wastes
4. Unsatisfactory disposal of radioactive waste
5. Industrial effluents and automobile exhausts.

But the above atmospheric pollutants appear relatively minor compared with *voluntary intake of three pollutants*—tobacco smoking, alcohol and intoxicant drugs. Attempts at prohibition of alcohol in some states in India or ban on smoking in some places have not been quite effective due to difficulty in implementation. Instead, prohibition has only resulted in off and on catastrophe of 'hooch tragedies' in some parts of this country due to illicit liquor consumption.

The present discussion on environmental and nutritional diseases is covered under the following groups:

1. *Environmental pollution:*
 - Air pollution
 - Tobacco smoking
2. *Chemical and drug injury:*
 - Therapeutic (iatrogenic) drug injury
 - Non-therapeutic toxic agents (e.g. alcohol, lead, carbon monoxide, drug abuse)

- Environmental chemicals

3. *Injury by physical agents:*

- Thermal and electrical injury
- Injury by ionising radiation

4. *Nutritional diseases:*

- Overnutrition (obesity)
- Undernutrition (starvation, protein energy malnutrition, vitamin deficiencies).

ENVIRONMENTAL POLLUTION

Any agent—chemical, physical or microbial, that alters the composition of environment is called pollutant. For survival of mankind, it is important to prevent depletion of ozone layer (O₃) in the outer space from pollutants such as chlorofluorocarbons and nitrogen dioxide produced in abundance by day-to-day activities on our planet earth due to industrial effluent and automobile exhausts.

AIR POLLUTION

A vast variety of pollutants are inhaled daily some of which may cause trivial irritation to the upper respiratory pathways, while others may lead to acute or chronic injury to the lungs, and some are implicated in causation of lung cancer. Whereas some pollutants are prevalent in certain industries (such as coal dust, silica, asbestos), others are general pollutants present widespread in the ambient atmosphere (e.g. sulphur dioxide, nitrogen dioxide, carbon monoxide). The latter group of environmental pollutants is acted upon by sunlight to produce secondary pollutants such as ozone and free radicals capable of oxidant cell injury to respiratory passages. In highly polluted cities where coal consumption and automobile exhaust accumulate in the atmosphere, the air pollutants become visible as 'smog'. It has been reported that 6 out of 10 largest cities in India have such severe air pollution problem that the annual level of suspended particles is about three times higher than the WHO standards. An estimated 50,000 persons die prematurely every year due to high level of pollution in these cities, of which 7,500 in Delhi alone.

The adverse effects of air pollutants on lung depend upon a few variables that include:

- longer duration of exposure;
- total dose of exposure;
- impaired ability of the host to clear inhaled particles; and
- particle size of 1-5 μm capable of getting impacted in the distal airways to produce tissue injury.

Pneumoconiosis is the group of lung diseases due to occupational over-exposure to pollutants.

TOBACCO SMOKING

Habits

Tobacco smoking is the most prevalent and preventable cause of disease and death. The harmful effects of smoking pipe and cigar are somewhat less. Long-term smokers of filter-tipped cigarettes appear to have 30-50% lower risk of development of cancer due to reduced inhalation of tobacco smoke constituents.

In India, a country of one billion people, a quarter (250 million) are tobacco users in one form or the other. Smoking *bidis* and chewing *pan masala*, *zarda* and *gutka* are more widely practiced than cigarettes (Fig. 9.1). Habit of smoking *chutta* (a kind of indigenous cigar) in which the lighted end is put in mouth is practiced in the Indian state of Andhra Pradesh and is associated with higher incidence of squamous cell carcinoma of hard palate. Another habit prevalent in Indian states of Uttar Pradesh and Bihar and in parts of Sri Lanka is chewing of tobacco alone or mixed with slaked lime as a bolus of *paan* kept in mouth for long hours which is the major cause of cancer of upper aerodigestive tract and oral cavity. *Hookah* smoking, in which tobacco smoke passes through a water-filled chamber which cools the smoke before it is inhaled by the smoker, is believed by some reports to deliver less tar and nicotine than cigarettes and hence fewer tobacco-related health consequences.

Besides the harmful effects of smoking on active smokers themselves, involuntary exposure of smoke to bystanders (passive smoking) is also injurious to health, particularly to infants and children.

Dose and Duration

The harmful effects of smoking are related to a variety of factors, the most important of which is dose of exposure expressed in terms of pack years. For example, one pack of cigarettes daily for 5 years means 5 pack years. It is estimated that a person who smokes 2 packs of cigarettes daily at the age of 30 years reduces his life by 8 years than a non-smoker. On cessation of smoking, the higher mortality slowly declines and the beneficial effect reaches the level of non-smokers after 20 or more of smoke-free years.

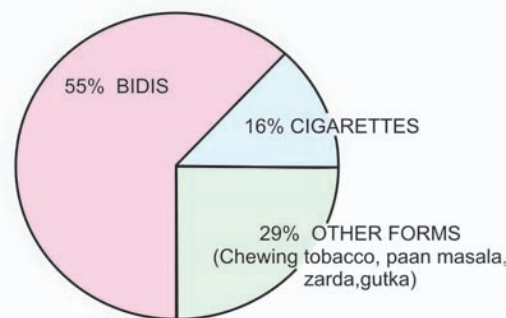


FIGURE 9.1

Consumption of tobacco in India as estimated by weight (Source: National Council of Applied Economic Research, New Delhi).

Tobacco-Related Diseases

Tobacco contains numerous toxic chemicals having adverse effects varying from minor throat irritation to carcinogenesis. Some of the important constituents of tobacco smoke with adverse effects are given in Table 9.1.

The major diseases accounting for higher mortality in tobacco smokers include, (in descending order of frequency):

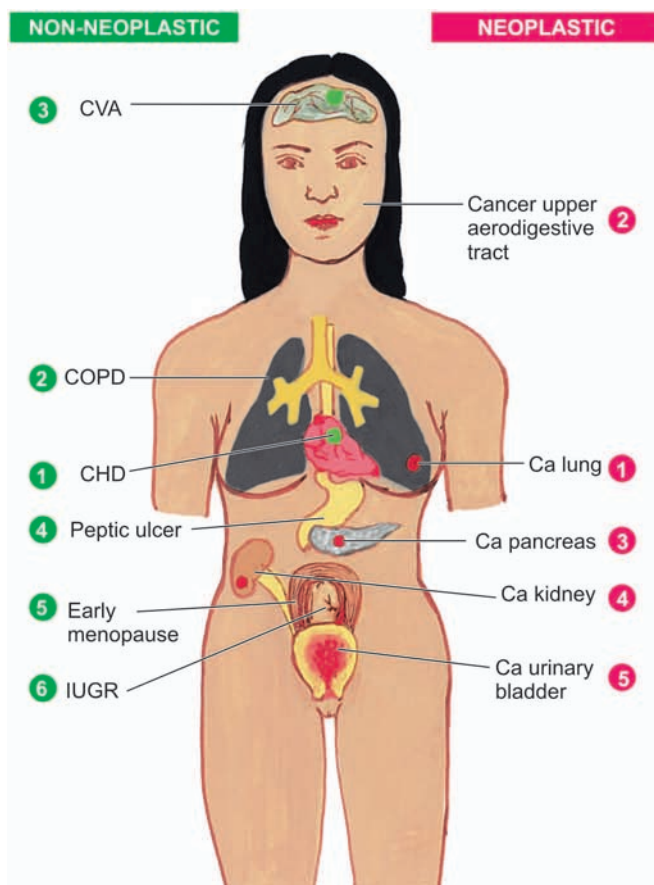
- Coronary heart disease;
- Cancer of the lung; and
- Chronic obstructive pulmonary disease (COPD).

Besides above, smokers suffer higher risk of development of a few other cancers and non-neoplastic conditions as illustrated in Fig. 9.2.

CORONARY HEART DISEASE. Cigarette smoking is one of the four major risk factors for myocardial infarction and acts synergistically with the other three—hypercholesterolaemia, hypertension and diabetes mellitus (Chapter 24). There is more severe, extensive and accelerated atherosclerosis of coronary arteries and

TABLE 9.1: Major Constituents of Tobacco Smoke with Adverse Effects.

ADVERSE EFFECT	CONSTITUENTS
1. <i>Carcinogenesis</i>	• Tar • Polycyclic aromatic hydrocarbons • Nitrosamines
2. <i>Tumour promoters</i>	• Nicotine • Phenol
3. <i>Irritation and toxicity to respiratory mucosa</i>	• Formaldehyde • Nitrogen oxide
4. <i>Reduced oxygen transport</i>	• Carbon monoxide

**FIGURE 9.2**

Major adverse effects of tobacco smoking. *Right side* shows smoking-related neoplastic diseases while *left side* indicates non-neoplastic diseases associated with smoking, numbered serially in order of frequency of occurrence.

aorta in smokers, possibly due to increased platelet aggregation and impaired lung function that causes reduced myocardial oxygen supply. Besides, the smokers have higher risk of development of atherosclerotic aortic aneurysm and Buerger's disease (thromboangiitis obliterans) affecting lower extremities.

LUNG CANCER. This is the most common cancer in men throughout world and most frequent cancer in women too in the United States exceeding in incidence beyond that of breast cancer in that country. Cigarette smoking is strongly implicated in evolution of lung cancer as described in Chapter 26.

OTHER CANCERS. Besides lung cancer, smokers have higher risk of development of cancer of upper aerodigestive tract (lips, oral cavity, larynx, oesophagus), pancreas, urinary bladder and kidney.

NON-NEOPLASTIC DISEASES. These include the following:

- i) Chronic obstructive pulmonary disease (COPD) that includes chronic bronchitis and emphysema as the most common.
- ii) Peptic ulcer disease with 70% higher risk in smokers.
- iii) Early menopause in smoker women.
- iv) In smoking pregnant women, higher risk of lower birth weight of foetus, higher perinatal mortality and intellectual deterioration of newborn.

CHEMICAL AND DRUG INJURY

During life, each one of us is exposed to a variety of chemicals and drugs. These are broadly divided into the following three categories:

- *Therapeutic (iatrogenic) agents* e.g. drugs, which when administered injudiciously are associated with adverse effects.
- *Non-therapeutic agents* e.g. alcohol, lead, carbon monoxide, drug abuse.
- *Environmental chemicals* e.g. long-term or accidental exposure to certain man-made or naturally-occurring chemicals.

THERAPEUTIC (IATROGENIC) DRUG INJURY

Though the basis of patient management is rational drug therapy, adverse drug reactions do occur in 2-5% of patients. In general, the risk of adverse drug reaction increases with increasing number of drugs administered. Adverse effects of drugs may appear due to:

- overdose;
- genetic predisposition;
- exaggerated pharmacologic response;
- interaction with other drugs; and
- unknown factors.

It is beyond the scope of this book to delve into the list of drugs with their harmful effects. However, some of the common forms of iatrogenic drug injury and the offending drugs are outlined in Table 9.2.

NON-THERAPEUTIC TOXIC AGENTS

ALCOHOLISM

Chronic alcoholism is defined as the regular imbibing of an amount of ethyl alcohol (ethanol) that is sufficient to harm an individual socially, psychologically or physically. It is difficult to give the number of 'drinks' after which the diagnosis of alcoholism can be made because of differences in individual susceptibility. However, adverse effects—acute as well as chronic, are related to the quantity of alcohol content imbibed and

TABLE 9.2: Iatrogenic Drug Injury.

ADVERSE EFFECT	OFFENDING DRUG
1. GASTROINTESTINAL TRACT	
<i>Gastritis, peptic ulcer</i>	Aspirin, nonsteroidal anti-inflammatory drugs (NSAIDs)
<i>Jejunal ulcer</i>	Enteric-coated potassium tablets
<i>Pancreatitis</i>	Thiazide diuretics
2. LIVER	
<i>Cholestatic jaundice</i>	Phenothiazines, tranquilizers, oral contraceptives
<i>Hepatitis</i>	Halothane, isoniazid
<i>Fatty change</i>	Tetracycline
3. NERVOUS SYSTEM	
<i>Cerebrovascular accidents</i>	Anticoagulants, Oral contraceptives
<i>Peripheral neuropathy</i>	Vincristine, antimalarials
<i>8th nerve deafness</i>	Streptomycin
4. SKIN	
<i>Acne</i>	Corticosteroids
<i>Urticaria</i>	Penicillin, sulfonamides
<i>Exfoliative dermatitis, Stevens-Johnson syndrome</i>	Penicillin, sulfonamides, phenyl butazone
<i>Fixed drug eruptions</i>	Chemotherapeutic agents
5. HEART	
<i>Arrhythmias</i>	Digitalis, propranolol
<i>Congestive heart failure</i>	Corticosteroids
<i>Cardiomyopathy</i>	Adriamycin
6. BLOOD	
<i>Aplastic anaemia</i>	Chloramphenicol
<i>Agranulocytosis, thrombocytopenia</i>	Antineoplastic drugs
<i>Immune haemolytic anaemia</i>	Penicillin
<i>Megaloblastic anaemia</i>	Methotrexate
7. LUNGS	
<i>Alveolitis, interstitial pulmonary fibrosis</i>	Anti-neoplastic drugs
<i>Asthma</i>	Aspirin, indomethacin
8. KIDNEYS	
<i>Acute tubular necrosis</i>	Gentamicin, kanamycin
<i>Nephrotic syndrome</i>	Gold salts
<i>Chronic interstitial nephritis, papillary necrosis</i>	Phenacetin, salicylates
9. METABOLIC EFFECTS	
<i>Hypercalcaemia</i>	Hypervitaminosis D, thiazide diuretics
<i>Hepatic porphyria</i>	Barbiturates
<i>Hyperuricaemia</i>	Anti-cancer chemotherapy
10. FEMALE REPRODUCTIVE TRACT	
<i>Cholelithiasis, thrombophlebitis, thromboembolism, benign liver cell adenomas</i>	Long-term use of oral contraceptives
<i>Vaginal adenosis, adenocarcinoma in daughters</i>	Diethylstilbesterol by pregnant women
<i>Foetal congenital anomalies</i>	Thalidomide in pregnancy

duration of consumption. Generally, 10 gm of ethanol is present in:

- a can of beer (or half a bottle of beer);
- 120 ml of neat wine; or
- 30 ml of 86° proof 43% liquor (small peg).

A daily consumption of 40 gm of ethanol (4 small pegs or 2 large pegs) is likely to be harmful but intake of 100 gm or more daily is certainly dangerous. Daily and heavy consumption of alcohol is more harmful than moderate social drinking since the liver, where ethanol is metabolised, gets time to heal.

Metabolism

Absorption of alcohol begins in the stomach and small intestine and appears in blood shortly after ingestion. Alcohol is then distributed to different organs and body fluids proportionate to the blood levels of alcohol. About 2-10% of absorbed alcohol is excreted via urine, sweat and exhaled through breath, the last one being the basis of breath test employed by law-enforcement agencies for alcohol. In brief, alcohol is metabolised in the liver by the following 3 pathways (Fig. 9.3):

- By the major rate-limiting pathway of alcohol dehydrogenase (ADH) in the cytosol, especially with low blood alcohol levels.
- Via microsomal P-450 system when the blood alcohol level is high.
- Minor pathway via catalase from peroxisomes.

In any of the three pathways, ethanol is biotransformed to toxic acetaldehyde in the liver and finally to carbon dioxide and water by acetyl coenzyme A.

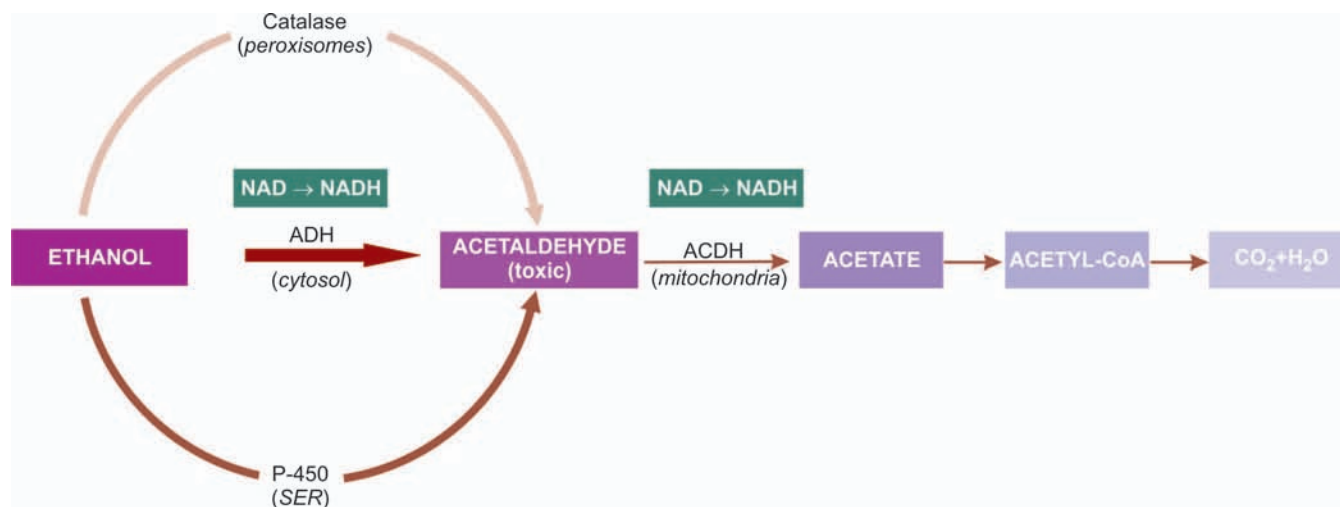
III-Effects of Alcoholism

Alcohol consumption in moderation and socially acceptable limits is practiced mainly for its mood-altering effects. Heavy alcohol consumption in unhabituated person is likely to cause acute ill-effects on different organs. Though the diseases associated with alcoholism are discussed in respective chapters later, the spectrum of ill-effects are outlined below.

A. ACUTE ALCOHOLISM. The acute effects of inebriation are most prominent on the central nervous system but it also injures the stomach and liver.

1. Central nervous system. Alcohol acts as a CNS depressant; the intensity of effects of alcohol on the CNS are related to the quantity consumed and duration over which consumed, which are reflected by blood levels of alcohol:

- The initial effect of alcohol is on subcortical structures which is followed by disordered cortical function, motor ataxia and behavioural changes. These changes

**FIGURE 9.3**

Metabolism of ethanol in the liver. Thickness and intensity of colour of arrow on left side of figure corresponds to extent of metabolic pathway followed (ADH=alcohol dehydrogenase; ACDH=hepatic acetaldehyde dehydrogenase; NAD=nicotinamide adenine dinucleotide; NADH=reduced NAD; NADP=nicotinamide adenine dinucleotide phosphate; NADPH=reduced NADP).

are apparent when blood alcohol levels do not exceed *100 mg/dl* which is the upper limit of sobriety in drinking as defined by law-enforcing agencies in most Western countries while dealing with cases of driving in drunken state.

■ Blood levels of *100-200 mg/dl* are associated with depression of cortical centres, lack of coordination, impaired judgement and drowsiness.

■ Stupor and coma supervene when blood alcohol levels are *about 300 mg/dl*.

■ Blood levels of alcohol *above 400 mg/dl* can cause anaesthesia, depression of medullary centre and death from respiratory arrest.

However, chronic alcoholics develop CNS tolerance and adaptation and, therefore, can withstand higher blood levels of alcohol without such serious effects.

2. Stomach. Acute alcohol intoxication may cause vomiting, acute gastritis and peptic ulceration.

3. Liver. Acute alcoholic injury to the liver results in alcoholic hepatitis.

B. CHRONIC ALCOHOLISM. Chronic alcoholism produces widespread injury to organs and systems. Contrary to the earlier belief that chronic alcoholic injury results from nutritional deficiencies, it is now known that most of the alcohol-related injury to different organs is due to toxic effects of alcohol and accumulation of its main toxic metabolite, acetaldehyde, in the blood. Other proposed mechanisms of tissue injury in chronic alcoholism is free-radical mediated injury and genetic susceptibility to alcohol-dependence and tissue damage.

Some of the more important organ effects in chronic alcoholism are as under (Fig. 9.4):

1. Liver. Alcoholic liver disease and cirrhosis are the most common and important effects of chronic alcoholism.

2. Pancreas. Chronic calcifying pancreatitis and acute pancreatitis are serious complications of chronic alcoholism.

3. Gastrointestinal tract. Gastritis, peptic ulcer and oesophageal varices associated with fatal massive bleeding may occur.

4. Central nervous system. Peripheral neuropathies and Wernicke-Korsakoff syndrome, cerebral atrophy, cerebellar degeneration and amblyopia (impaired vision) are seen in chronic alcoholics.

5. Cardiovascular system. Alcoholic cardiomyopathy and beer-drinkers' myocardiosis with consequent dilated cardiomyopathy may occur. Level of HDL (atherosclerosis-protective lipoprotein), however, has been shown to increase with moderate consumption of alcohol.

6. Endocrine system. In men, testicular atrophy, feminisation, loss of libido and potency, and gynaecomastia may develop. These effects appear to be due to lowering of testosterone levels.

7. Blood. Haematopoietic dysfunction with secondary megaloblastic anaemia and increased red blood cell volume may occur.

8. Immune system. Alcoholics are more susceptible to various infections.

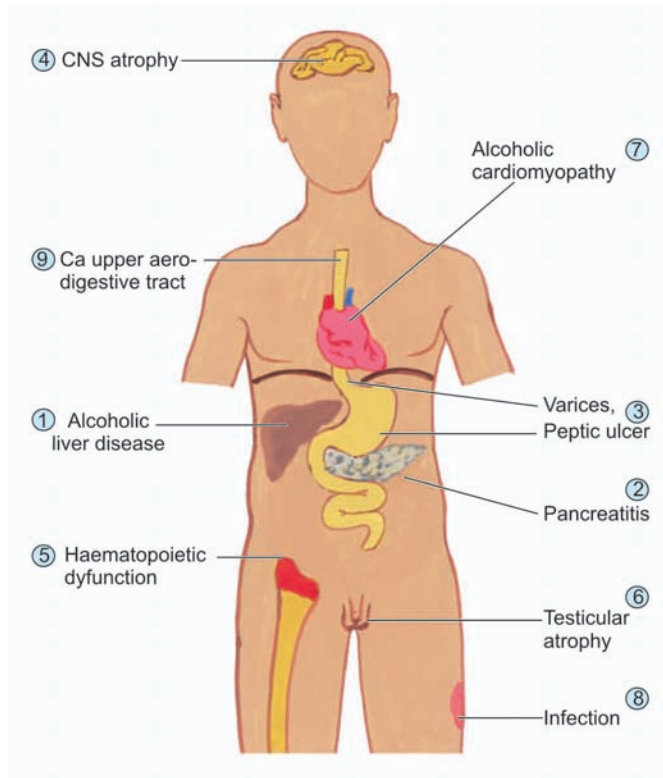


FIGURE 9.4

Complications of chronic alcoholism.

9. Cancer. There is higher incidence of cancers of upper aerodigestive tract in chronic alcoholics but the mechanism is not clear.

LEAD POISONING

Lead poisoning may occur in children or adults due to accidental or occupational ingestion.

In children, the main sources are:

- Chewing of lead-containing furniture items, toys or pencils.
- Eating of lead paint flakes from walls.

In adults, the sources are:

- Occupational exposure to lead during spray painting, recycling of automobile batteries (lead oxide fumes), mining, and extraction of lead.
- Accidental exposure by contaminated water supply, house freshly coated with lead paint, and sniffing of lead-containing petrol (hence unleaded petrol introduced as fuel).

Lead is absorbed through the gastrointestinal tract or lungs. The absorbed lead is distributed in two types of tissues (Fig. 9.5):

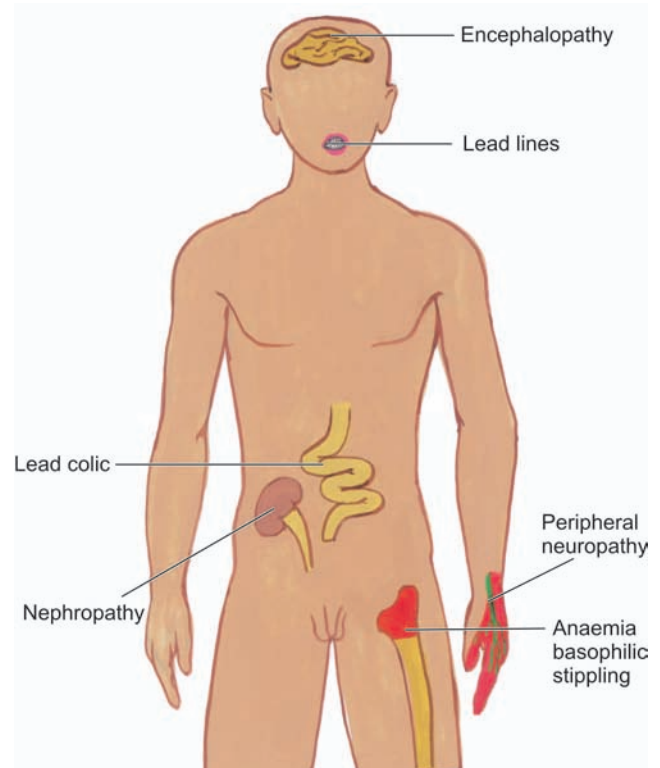


FIGURE 9.5

Complications of lead poisoning.

a) *Bones, teeth, nails and hair* representing relatively harmless pool of lead. About 90% of absorbed lead accumulates in the developing metaphysis of bones in children and appears as areas of increased bone densities ('lead lines') on X-ray. Lead lines are also seen in the gingiva.

b) *Brain, liver, kidneys and bone marrow* accumulate the remaining 10% lead which is directly toxic to these organs. It is excreted via kidneys.

Lead toxicity occurs in the following organs predominantly:

1. Nervous system: The changes are as under:

- *In children*, lead encephalopathy; oedema of brain, flattening of gyri and compression of ventricles.
- *In adults*, demyelinating peripheral motor neuropathy which typically affects radial and peroneal nerves resulting in wrist drop and foot drop respectively.

2. Haematopoietic system: The changes in blood are quite characteristic:

- *Microcytic hypochromic anaemia* due to inhibition of two enzymes: delta-aminolevulinic acid dehydrogenase required for haem synthesis, and through inhibition of

ferroketolase required for incorporation of ferrous iron into the porphyrin ring.

■ Prominent basophilic stippling of erythrocytes.

3. **Kidneys:** Lead is toxic to proximal tubular cells of the kidney and produces *lead nephropathy* characterised by accumulation of intranuclear inclusion bodies consisting of lead-protein complex in the proximal tubular cells.

4. **Gastrointestinal tract:** Lead toxicity in the bowel manifests as acute abdomen presenting as lead colic.

CARBON MONOXIDE POISONING

Carbon monoxide (CO) is a colourless and odourless gas produced by incomplete combustion of carbon. Sources of CO gas are:

- automobile exhaust;
- burning of fossil fuel in industries or at home; and
- tobacco smoke.

CO is an important cause of accidental death due to systemic oxygen deprivation of tissues. This is because haemoglobin has about 200-times higher affinity for CO than for O₂ and thus varying amount of carboxyhaemoglobin is formed depending upon the extent of CO poisoning. Besides, carboxyhaemoglobin interferes with the release of O₂ from oxyhaemoglobin causing further aggravation of tissue hypoxia. Diagnosis of CO poisoning is, therefore, best confirmed by carboxyhaemoglobin levels in the blood.

CO poisoning may present in 2 ways:

■ **Acute CO poisoning** in which there is sudden development of brain hypoxia characterised by oedema and petechial haemorrhages.

■ **Chronic CO poisoning** presents with nonspecific changes of slowly developing hypoxia of the brain.

DRUG ABUSE

Drug abuse is defined as the use of certain drugs for the purpose of 'mood alteration' or 'euphoria' or 'kick' but subsequently leading to habit-forming, dependence and eventually addiction. Some of the commonly abused drugs and substances are as under:

1. *Marijuana* or '*pot*' is psychoactive substance most widely used. It is obtained from the leaves of the plant *Cannabis sativa* and contains tetrahydrocannabinol (THC). It may be smoked or ingested.
2. *Derivatives of opium* that includes heroin and morphine. Opioids are derived from the poppy plant. Heroin and morphine are self-administered intravenously or subcutaneously.

3. *CNS depressants* include barbiturates, tranquilisers and alcohol.

4. *CNS stimulants* e.g. cocaine and amphetamines.

5. *Psychedelic drugs* (meaning enjoyable perception-giving) e.g. LSD.

6. *Inhalants* e.g. glue, paint thinner, nail polish remover, aerosols, amyl nitrite.

It is beyond the scope of the present discussion to go into the pharmacologic actions of all these substances. However, apart from pharmacologic and physiologic actions of these street drugs, the most common complication is introduction of infection by parenteral use of many of these drugs. Sharing of needles by the drug-addicts accounts for high risk of most feared viral infections in them, AIDS and viral hepatitis (both HBV and HCV). A few common drug abuse-related infectious complications are:

1. At the site of injection—cellulitis, abscesses, ulcers, thrombosed veins
2. Thrombophlebitis
3. Bacterial endocarditis
4. High risk for AIDS
5. Viral hepatitis and its complications
6. Focal glomerulonephritis
7. Talc (foreign body) granuloma formation in the lungs.

ENVIRONMENTAL CHEMICALS

A large number of chemicals are found as contaminants in the ecosystem, food and water supply and find their way into the food chain of man. These substances exert their toxic effects depending upon their mode of absorption, distribution, metabolism and excretion. Some of the substances are directly toxic while others cause ill-effects via their metabolites. Environmental chemicals may have slow damaging effect or there may be sudden accidental exposure such as the Bhopal gas tragedy in India due to accidental leakage of methyl isocyanate (MIC) gas in December 1984.

Some of the common examples of environmental chemicals are given below:

1. **Agriculture chemicals.** Modern agriculture thrives on pesticides, fungicides, herbicides and organic fertilisers which may pose a potential acute poisoning as well as long-term hazard. The problem is particularly alarming in developing countries like India, China and Mexico where farmers and their families are unknowingly exposed to these hazardous chemicals during aerial spraying of crops.

■ Acute poisoning by organophosphate insecticides is too well known as accidental or suicidal poison by inhibiting acetyl cholinesterase and sudden death.

■ **Chronic human exposure** to low level agricultural chemicals is implicated in cancer, chronic degenerative diseases, congenital malformations and impotence but the exact cause-and-effect relationship is lacking.

According to the WHO estimates, about 7.5 lakh people are taken ill every year worldwide with pesticide poisoning, half of which occur in the third world countries due to use of hazardous pesticides which are otherwise banned in advanced countries. Pesticide residues in food items such as in fruits, vegetables, cereals, grains, pulses etc is of greatest concern. According to a country-wide study conducted by the Indian Council of Medical Research (ICMR), New Delhi, recently, it has been found that 82% of milk samples from 12 different Indian states contained residues of pesticides above the tolerance limit such as BHC and DDT which are widely used insecticides in India.

2. Volatile organic solvents. Volatile organic solvents and vapours are used in industry quite commonly and their exposure may cause acute toxicity or chronic hazard, often by inhalation than by ingestion. Such substances include methanol, chloroform, petrol, kerosene, benzene, ethylene glycol, toluene etc.

3. Metals. Pollution by occupational exposure to toxic metals such as mercury, arsenic, cadmium, iron, nickel and aluminium are important hazardous environmental chemicals.

4. Aromatic hydrocarbons. The halogenated aromatic hydrocarbons containing polychlorinated biphenyl which are contaminant in several preservatives, herbicides and antibacterial agents are a chronic health hazard.

5. Cyanide. Cyanide in the environment is released by combustion of plastic, silk and is also present in cassava and the seeds of apricots and wild cherries. Cyanide is a very toxic chemical and kills by blocking cellular respiration by binding to mitochondrial cytochrome oxidase.

6. Environmental dusts. These substances cause pneumoconioses while those implicated in cancer.

INJURY BY PHYSICAL AGENTS

THERMAL AND ELECTRICAL INJURY

Thermal and electrical burns, fall in body temperature below 35°C (hypothermia) and elevation of body temperature above 41°C (hyperthermia), are all associated with tissue injury.

■ **Hypothermia** may cause focal injury as in frostbite, or systemic injury and death as occurs on immersion in cold water for varying time.

■ **Hyperthermia** likewise, may be localised as in cutaneous burns, and systemic as occurs in fevers.

■ **Thermal burns** depending upon severity are categorised into full thickness (third degree) and partial thickness (first and second degree). The most serious complications of burns are haemoconcentration, infections and contractures on healing.

■ **Electrical burns** may cause damage by *firstly*, electrical dysfunction of the conduction system of the heart and death by ventricular fibrillation, and *secondly* by heat produced by electrical energy.

INJURY BY RADIATION

As discussed in the preceding chapter, the most important form of radiation injury is ionising radiation which has three types of effects on cells:

- i) Somatic effects which cause acute cell killing.
- ii) Genetic damage by mutations and therefore, causes genetic defects.
- iii) Malignant transformation of cells (Chapter 21).

Ionising radiation is widely employed for diagnostic purpose as well as for radiotherapy of malignant tumours. Radiation-induced cell death is mediated by radiolysis of water in the cell with generation of toxic hydroxyl radicals (page 32). During radiotherapy, some normal cells coming in the field of radiation are also damaged. In general, radiation-induced tissue injury predominantly affects endothelial cells of small arteries and arterioles, causing necrosis and ischaemia.

Ionising radiation causes damage to the following major organs:

1. *Skin*: radiation dermatitis, cancer.
2. *Lungs*: interstitial pulmonary fibrosis.
3. *Heart*: myocardial fibrosis, constrictive pericarditis.
4. *Kidney*: radiation nephritis.
5. *Gastrointestinal tract*: strictures of small bowel and oesophagus.
6. *Gonads*: testicular atrophy in males and destruction of ovaries.
7. *Haematopoietic tissue*: pancytopenia due to bone marrow depression.
8. *Eyes*: cataract.

Besides ionising radiation, other form of harmful radiation is *solar (u.v.) radiation* which may cause acute skin injury as sunburns, chronic conditions such as solar keratosis and early onset of cataracts in the eyes. It may, however, be mentioned in passing here that electromagnetic radiation produced by microwaves (ovens, radars, diathermy) or ultrasound waves used for diagnostic purposes do not produce ionisation and thus are not known to cause any tissue injury.

NUTRITIONAL DISEASES

Nutritional status of a society varies according to the socioeconomic conditions. In the Western world, nutritional imbalance is more often a problem accounting for increased frequency of obesity, while in developing countries of Africa, Asia and South America, chronic malnutrition is a serious problem, particularly in children.

Before describing the nutritional diseases, it is essential to know the components of normal and adequate nutrition. The nutrients in diet can be grouped into essential and non-essential.

ESSENTIAL NUTRIENTS. There are 6 basic groups of essential nutrients. These are as under:

1. Proteins. Dietary proteins provide the body with amino acids for endogenous protein synthesis and are also a metabolic fuel for energy (1 g of protein provides 4 Kcal). *Eight essential amino acids* (isoleucine, leucine, lysine, methionine, phenylalanine, theonine, tryptophan and valine) as well as histidine must be supplied by dietary intake as these cannot be synthesised in the body.

2. Fats. Fats and fatty acids (in particular linolenic, linoleic and arachidonic acid) should comprise about 35% of diet with an increased ratio of poly-unsaturated to saturated fats so as to minimise the risk of atherosclerosis (1 g of fat yields 9 Kcal).

3. Carbohydrates. Dietary carbohydrates, though not essential, are the major source of dietary calories (1 g of carbohydrate provides 4 Kcal).

4. Vitamins. These are mainly derived from exogenous dietary sources and are essential for maintaining the normal structure and function of cells. Vitamin deficiencies result in individual deficiency syndromes, or may be part of a multiple deficiency state.

5. Minerals. A number of minerals like iron, calcium, phosphorus and certain trace elements (e.g. zinc, copper, selenium, iodine, chlorine, sodium, potassium, magnesium, manganese, cobalt, molybdenum etc) are essential for health. Their deficiencies result in a variety of lesions and deficiency syndromes.

6. Water. Water intake is essential to cover the losses in faeces, urine, exhalation and insensible loss so as to avoid under- or over-hydration.

NON-ESSENTIAL NUTRIENTS. Dietary fibre composed of cellulose, hemicellulose and pectin, though considered non-essential, are important due to beneficial effects in colonic cancer, diabetes, coronary artery disease, and in lowering the incidence of colonic cancer.

Pathogenesis of Deficiency Diseases

The nutritional deficiency disease develops when the essential nutrients are not provided to the cells adequately. The nutritional deficiency may be of 2 types:

1. Primary deficiency. This is due to either the lack or decreased amount of essential nutrients in diet.

2. Secondary or conditioned deficiency. Secondary or conditioned deficiency is malnutrition occurring as a result of the various factors. These are as under:

i) *Interference with ingestion* e.g. in gastrointestinal disorders, neuropsychiatric illness, anorexia, alcoholism, food allergy, pregnancy.

ii) *Interference with absorption* e.g. in hypermotility of the gut, achlorhydria, biliary disease.

iii) *Interference with utilisation* e.g. in liver dysfunction, malignancy, hypothyroidism.

iv) *Increased excretion* e.g. in lactation, perspiration, polyuria.

v) *Increased nutritional demand* e.g. in fever, pregnancy, lactation, hyperthyroidism.

Irrespective of the type of nutritional deficiency (primary or secondary), nutrient reserves in the tissues begin to get depleted, which initially result in biochemical alterations and eventually lead to functional and morphological changes in tissues and organs (Fig. 9.6).

In the following pages, a brief account of nutritional imbalance (viz. *obesity*) is followed by description of multiple or mixed deficiencies (viz. *starvation*, *protein-energy malnutrition*) and individual nutrient deficiencies (viz. *vitamin deficiencies*).

OBESITY

Dietary imbalance and overnutrition may lead to diseases like obesity. Aesthetic considerations aside, *obesity is defined as an excess of adipose tissue that imparts health risk; a body weight of 20% excess over ideal weight for age, sex and height is considered a health risk.*

ETIOLOGY. *Obesity results when caloric intake exceeds utilisation.* The imbalance of these two components can occur in the following situations:

1. *Inadequate pushing of oneself away from the dining table causing overeating.*

2. *Insufficient pushing of oneself out of the chair leading to inactivity and sedentary life style.*

3. *Genetic predisposition to develop obesity.*

4. *Diets largely derived from carbohydrates and fats than protein-rich diet.*

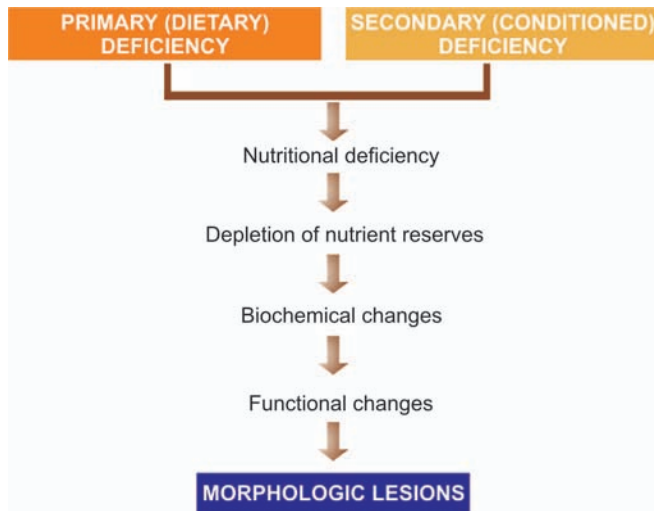


FIGURE 9.6

Schematic representation of pathogenesis of nutritional deficiency diseases.

5. *Secondary obesity* may result following a number of underlying diseases such as hypothyroidism, Cushing's disease, insulinoma and hypothalamic disorders.

SEQUELAE OF OBESITY. Marked obesity is a serious health hazard and may predispose to a number of clinical disorders and pathological changes described below and illustrated in Fig. 9.7.

PATHOLOGIC CHANGES. Obesity is associated with increased adipose stores in the subcutaneous tissues, skeletal muscles, internal organs such as the kidneys, heart, liver and omentum; fatty liver is also more common in obese individuals. There is increase in both size and number of adipocytes i.e. there is hypertrophy as well as hyperplasia.

METABOLIC CHANGES. These are as under:

1. **Hyperinsulinaemia.** Increased insulin secretion is a feature of obesity. Many obese patients exhibit hyperglycaemia or frank diabetes despite hyperinsulinaemia. This is due to a state of insulin-resistance consequent to tissue insensitivity.
2. **Non-insulin dependent diabetes.** There is a strong association of non-insulin dependent diabetes mellitus with obesity. Obesity often exacerbates the diabetic state and in many cases weight reduction often leads to amelioration of diabetes.
3. **Hypertension.** A strong association between hypertension and obesity is observed which is perhaps due

to increased blood volume. Weight reduction leads to significant reduction in systolic blood pressure.

4. **Hyperlipoproteinaemia.** The plasma cholesterol circulates in the blood as low density lipoprotein (LDL) containing most of the circulating triglycerides. Obesity is strongly associated with VLDL and mildly with LDL. Total blood cholesterol levels are also elevated in obesity.

5. **Atherosclerosis.** Obesity predisposes to development of atherosclerosis.

6. **Coronary artery disease and stroke.** As a result of atherosclerosis and hypertension, there is increased risk of myocardial infarction and stroke in obese individuals.

7. **Cholelithiasis.** There is six times higher incidence of gallstones in obese persons, mainly due to increased total body cholesterol.

8. **Hypoventilation syndrome (Pickwickian syndrome).** This is characterised by hypersomnolence, both

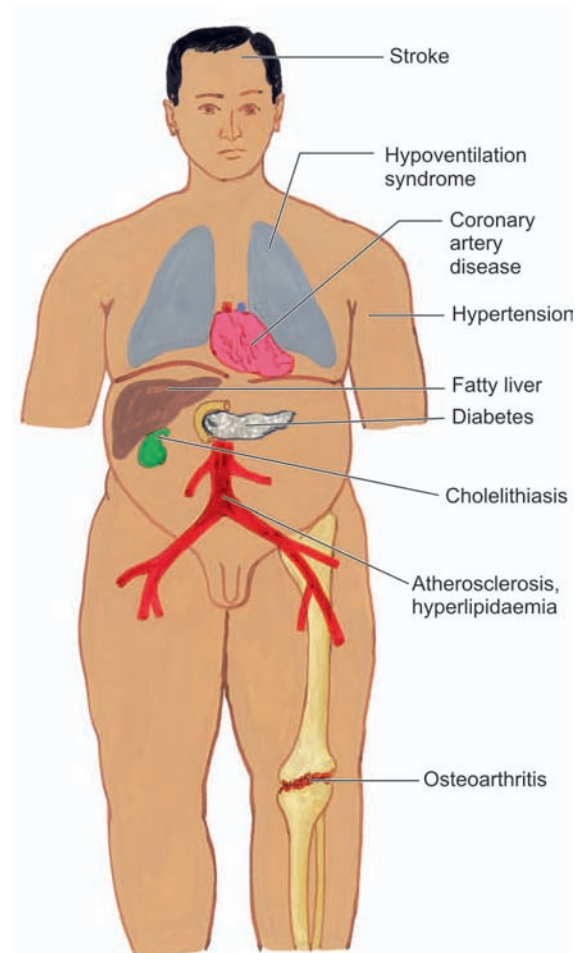


FIGURE 9.7

Major sequelae of obesity.

at night and during day in obese individuals along with carbon dioxide retention, hypoxia, polycythaemia and eventually right-sided heart failure. (Mr Pickwick was a character, the fat boy, in Charles Dickens' *Pickwick Papers*. The term pickwickian syndrome was first used by Sir William Osler for the sleep-apnoea syndrome).

9. Osteoarthritis. These individuals are more prone to develop degenerative joint disease due to wear and tear following trauma to joints as a result of large body weight.

10. Cancer. Certain cancers such as of endometrium and breast seem to be related to obesity. Particularly implicated are the diets derived from animal fats and meats. However, a definite evidence in support of role of diet in cancer is lacking.

STARVATION

Starvation is a state of overall deprivation of nutrients. Its causes may be:

- i) deliberate fasting—religious or political;
- ii) famine conditions in a country or community; or
- iii) secondary undernutrition such as due to chronic wasting diseases (infections, inflammatory conditions, liver disease), cancer etc. Cancer results in malignant cachexia as a result of which cytokines are elaborated e.g. tumour necrosis factor- α , elastases, proteases etc.

A starved individual has lax, dry skin, wasted muscles and atrophy of internal organs.

METABOLIC CHANGES. The following metabolic changes take place in starvation:

1. Glucose. *Glucose stores* of the body are sufficient for one day's metabolic needs only. During fasting state, insulin-independent tissues such as the brain, blood cells and renal medulla continue to utilise glucose while insulin-dependent tissues like muscle stop taking up glucose. This results in release of glycogen stores of the liver to maintain normal blood glucose level. Subsequently, hepatic gluconeogenesis from other sources such as breakdown of proteins takes place.

2. Proteins. Protein stores and the triglycerides of adipose tissue have enough energy for about 3 months in an individual. *Proteins* breakdown to release amino acids which are used as fuel for hepatic gluconeogenesis so as to maintain glucose needs of the brain. This results in nitrogen imbalance due to excretion of nitrogen compounds as urea.

3. Fats. After about one week of starvation, protein breakdown is decreased while *triglycerides* of adipose tissue breakdown to form glycerol and fatty acids. The fatty acids are converted into ketone bodies in the liver

which are used by most organs including brain in place of glucose. Starvation can then continue till all the body fat stores are exhausted following which death occurs.

PROTEIN-ENERGY MALNUTRITION

The inadequate consumption of protein and energy as a result of primary dietary deficiency or conditioned deficiency may cause loss of body mass and adipose tissue, resulting in protein energy or protein calorie malnutrition (PEM or PCM). The primary deficiency is more frequent due to socioeconomic factors limiting the quantity and quality of dietary intake, particularly prevalent in the developing countries of Africa, Asia and South America. The impact of deficiency is marked in infants and children.

The spectrum of *clinical syndromes* produced as a result of PEM includes the following (Fig. 9.8):

1. *Kwashiorkor* which is related to protein deficiency though calorie intake may be sufficient.
2. *Marasmus* is starvation in infants occurring due to overall lack of calories.

The salient features of the two conditions are contrasted in Table 9.3. However, it must be remembered that mixed forms of kwashiorkor-marasmus syndrome may also occur.

DISORDERS OF VITAMINS

Vitamins are organic substances which can not be synthesised within the body and are essential for maintenance of normal structure and function of cells. Thus, these substances must be provided in the human diet. Most of the vitamins are of plant or animal origin so that they normally enter the body as constituents of ingested plant food or animal food. They are required in minute amounts in contrast to the relatively large amounts of essential amino acids and essential fatty acids. Vitamins do not play any part in production of energy.

In the developing countries, *multiple deficiencies* of vitamins and other nutrients are common due to generalised malnutrition of dietary origin. In the developed countries, *individual vitamin deficiencies* are noted more often, particularly in children, adolescent, pregnant and lactating women, and in some due to poverty. However, secondary or conditioned deficiencies can occur in either case. Chronic alcoholism comprises an important cause of vitamin deficiency in the United States.

Vitamins are conventionally divided into 2 groups: fat-soluble and water-soluble.

1. Fat-soluble vitamins are vitamin A, D, E and K. They are absorbed from intestine in the presence of bile

TABLE 9.3: Contrasting Features of Kwashiorkor and Marasmus.

FEATURE	KWASHIORKOR	MARASMUS
Definition	Protein deficiency with sufficient calorie intake	Starvation in infants with overall lack of calories
Clinical features (Fig. 9.8)	Occurs in children between 6 months and 3 years of age Growth failure Wasting of muscles but preserved adipose tissues Oedema, localised or generalised, present Enlarged fatty liver Serum proteins low Anaemia present 'Flag sign'—alternate bands of light (depigmented) and dark (pigmented) hair	Common in infants under 1 year of age Growth failure Wasting of all tissues including muscles and adipose tissues Oedema absent No hepatic enlargement Serum proteins low Anaemia present Monkey-like face, protuberant abdomen, thin limbs
Morphology	Enlarged fatty liver Atrophy of different tissues and organs but subcutaneous fat preserved	No fatty liver Atrophy of different tissues and organs including subcutaneous fat

salts and intact pancreatic function. Their deficiencies occur more readily due to conditioning factors (*secondary deficiency*). Beside the deficiency syndromes of these vitamins, a state of *hypervitaminosis* due to excess of vitamin A and D also occurs.

2. Water-soluble vitamins are vitamin C and B complex group. These vitamins are more readily absorbed from small intestine. Deficiency of these vitamins is mainly due to *primary (dietary) factors*. Being water soluble, these vitamins are more easily lost due to cooking or processing of food.

Table 9.4 sums up the various clinical disorders produced by vitamin deficiencies.

FAT-SOLUBLE VITAMINS

Vitamin A (Retinol)

PHYSIOLOGY. Vitamin A or retinol is a fat soluble alcohol. It is available in diet in 2 forms:

- As *preformed retinol*, the dietary sources of which are animal-derived foods such as yolk of eggs, butter, whole milk, fish, liver, kidney.
- As *provitamin precursor carotenoid*, which is derived from β -carotene-containing foods such as yellow plants and vegetables e.g. carrots, potatoes, pumpkins, mangoes, spinach. β -carotene can be absorbed intact or

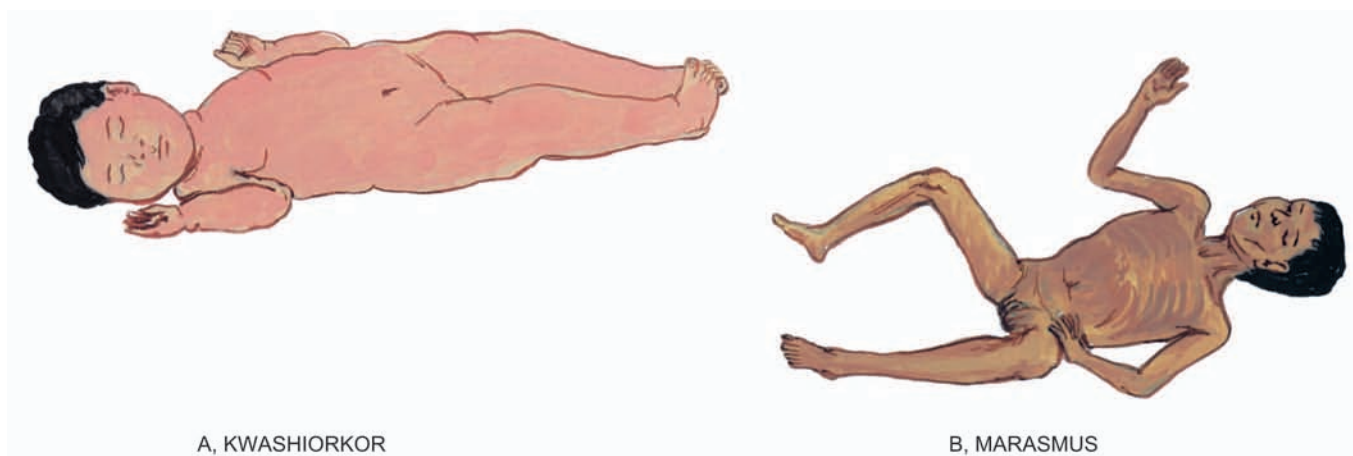


FIGURE 9.8

Two forms of PEM.

TABLE 9.4: Vitamin Deficiencies.

VITAMINS	DEFICIENCY DISORDERS
I. FAT-SOLUBLE VITAMINS	
<i>Vitamin A</i> (<i>Retinol</i>)	Ocular lesions (night blindness, xerophthalmia, keratomalacia, Bitot's spots, blindness) Cutaneous lesions (xeroderma) Other lesions (squamous metaplasia of respiratory epithelium, urothelium and pancreatic ductal epithelium, subsequent anaplasia; retarded bone growth)
<i>Vitamin D</i> (<i>Calcitriol</i>)	Rickets in growing children Osteomalacia in adults Hypocalcaemic tetany
<i>Vitamin E</i>	Degeneration of neurons, retinal pigments, axons of peripheral nerves; denervation of muscles Reduced red cell life span Sterility in male and female animals
<i>Vitamin K</i>	Hypoproteinaemia (in haemorrhagic disease of newborn, biliary obstruction, malabsorption, anticoagulant therapy, anti-biotic therapy, diffuse liver disease)
II. WATER-SOLUBLE VITAMINS	
<i>Vitamin C</i> (<i>Ascorbic acid</i>)	Scurvy (haemorrhagic diathesis, skeletal lesions, delayed wound healing, anaemia, lesions in teeth and gums)
<i>Vitamin B Complex</i>	
(i) <i>Thiamine</i> (<i>Vitamin B₁</i>)	Beriberi ('dry' or peripheral neuritis, 'wet' or cardiac manifestations, 'cerebral' or Wernicke-Korsakoff's syndrome)
(ii) <i>Riboflavin</i> (<i>Vitamin B₂</i>)	Ariboflavinosis (ocular lesions, cheilosis, glossitis, dermatitis)
(iii) <i>Niacin</i> (<i>Nicotinic acid</i>)	Pellagra (dermatitis, diarrhoea, dementia)
(iv) <i>Pyridoxine</i> (<i>Vitamin B₆</i>)	Vague lesions (convulsions in infants, dermatitis, cheilosis, glossitis, sideroblastic anaemia)
(v) <i>Folate</i> (<i>Folic acid</i>)	Megaloblastic anaemia
(vi) <i>Cyanocobalamin</i> (<i>Vitamin B₁₂</i>)	Megaloblastic anaemia Pernicious anaemia

converted in the intestinal mucosa to form retinaldehyde which is subsequently reduced to retinol.

Retinol is stored in the liver cells and released for transport to peripheral tissues after binding to retinol-binding protein found in blood.

The **physiologic functions** of retinol are as follows:

1. *Maintenance of normal vision in reduced light.* This involves synthesis of rhodopsin, a light sensitive pigment in the rods and cones of retina, by oxidation of retinol. This pigment then transforms the radiant energy into nerve impulses.
2. *Maintenance of structure and function of specialised epithelium.* Retinol plays an important role in the synthesis of glycoproteins of the cell membrane of specialised epithelium such as mucus-secreting columnar epithelium in glands and mucosal surfaces, respiratory epithelium and urothelium.

3. *Maintenance of normal cartilaginous and bone growth.*

4. *Anti-proliferative effect.* Recently, it has been found that β -carotene by anti-oxidant action may cause regression of certain non-tumorous skin diseases, premalignant conditions and certain cancers.

LESIONS IN VITAMIN A DEFICIENCY. Nutritional deficiency of vitamin A is common in countries of South-East Asia, Africa, Central and South America whereas malabsorption syndrome may account for conditioned vitamin A deficiency in developed countries.

PATHOLOGIC CHANGES. Consequent to vitamin A deficiency following pathologic changes are seen (Fig. 9.9):

1. **Ocular lesions.** Lesions in the eyes are most obvious. *Night blindness* is usually the first sign of

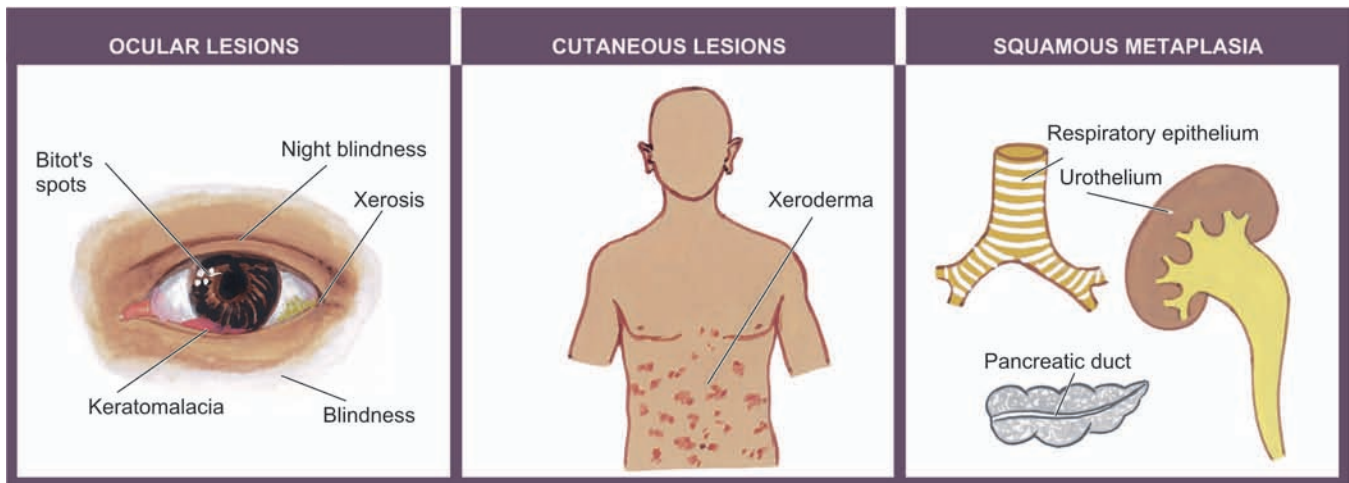


FIGURE 9.9

Lesions resulting from vitamin A deficiency.

vitamin A deficiency. As a result of replacement metaplasia of mucus-secreting cells by squamous cells, there is dry and scaly scleral conjunctiva (*xerophthalmia*). The lacrimal duct also shows hyperkeratosis. Corneal ulcers may occur which may get infected and cause *keratomalacia*. *Bitot's spots* may appear which are focal triangular areas of opacities due to accumulation of keratinised epithelium. If these occur on cornea, they impede transmission of light. Ultimately, infection, scarring and opacities lead to *blindness*.

2. Cutaneous lesions. The skin develops papular lesions giving toad-like appearance (*xeroderma*). This is due to follicular hyperkeratosis and keratin plugging in the sebaceous glands.

3. Other lesions. These are as under:

- i) *Squamous metaplasia of respiratory epithelium* of bronchus and trachea may predispose to respiratory infections.
- ii) *Squamous metaplasia of pancreatic ductal epithelium* may lead to obstruction and cystic dilatation.
- iii) *Squamous metaplasia of urothelium* of the pelvis of kidney may predispose to pyelonephritis and perhaps to renal calculi.
- iv) Long-standing metaplasia may cause progression to *anaplasia* under certain circumstances.
- v) *Bone growth* in vitamin A deficient animals is retarded.

HYPERVITAMINOSIS A. Very large doses of vitamin A can produce toxic manifestations in children as well as in adults. These may be *acute* or *chronic*.

Acute toxicity. This results from a single large dose of vitamin A. The effects include neurological manifestations resembling brain tumour e.g. headache, vomiting, stupor, papilloedema.

Chronic toxicity. The clinical manifestations of chronic vitamin A excess are as under:

- i) *Neurological* such as severe headache and disordered vision due to increased intracranial pressure.
- ii) *Skeletal pains* due to loss of cortical bone by increased osteoclastic activity as well as due to exostosis.
- iii) *Cutaneous involvement* may be in the form of pruritus, fissuring, sores at the corners of mouth and coarseness of hair.
- iv) *Hepatomegaly* with parenchymal damage and fibrosis.
- v) *Hypercarotenaemia* is yellowness of palms and skin due to excessive intake of β -carotene containing foods like carrots or due to inborn error of metabolism.

The effects of toxicity usually disappear on stopping excess of vitamin A intake.

Vitamin D (Calcitriol)

PHYSIOLOGY. This fat-soluble vitamin exists in 2 activated *sterol forms*:

- Vitamin D₂ or *calciferol*; and
- Vitamin D₃ or *cholecalciferol*.

The material originally described as vitamin D₁ was subsequently found to be impure mixture of sterols. Since vitamin D₂ and D₃ have similar metabolism and functions, they are therefore referred to as vitamin D.

There are 2 *main sources* of vitamin D:

- i) **Endogenous synthesis.** 80% of body's need of vitamin D is met by endogenous synthesis from the

action of ultraviolet light on 7-dehydrocholesterol widely distributed in oily secretions of the skin. The vitamin so formed by irradiation enters the body directly through the skin. Pigmentation of the skin reduces the beneficial effects of ultraviolet light.

ii) Exogenous sources. The other source of vitamin D is diet such as deep sea fish, fish oil, eggs, butter, milk, some plants and grains.

Irrespective of the source of vitamin D, it must be converted to its *active metabolites* (25-hydroxy vitamin D and 1,25-dihydroxy vitamin D or calcitriol) for being functionally active (Fig. 9.10).

1, 25-dihydroxy vitamin D (calcitriol) is 5-10 times more potent biologically than 25-hydroxy vitamin D. The production of calcitriol by the kidney is regulated by:

- plasma levels of calcitriol (hormonal feedback);
- plasma calcium levels (hypocalcaemia stimulates synthesis); and
- plasma phosphorus levels (hypophosphataemia stimulates synthesis).

The main storage site of vitamin D is the adipose tissue rather than the liver which is the case with vitamin A.

The main **physiologic functions** of the most active metabolite of vitamin D, calcitriol, are as follows:

1. Maintenance of normal plasma levels of calcium and phosphorus. The major essential function of vitamin D is to promote mineralisation of bone. This is achieved by the following actions of vitamin D:

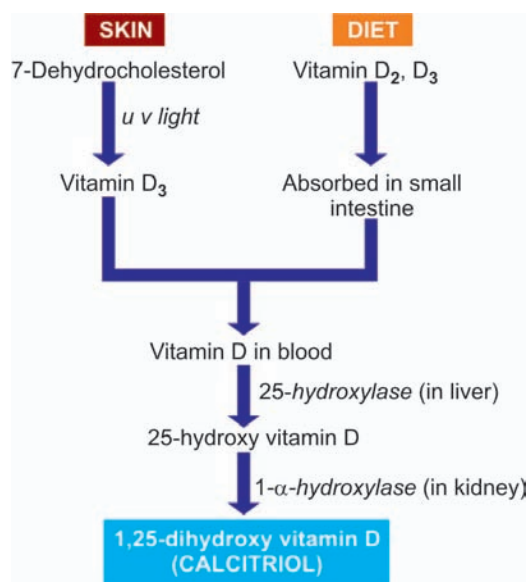


FIGURE 9.10

Normal metabolism of vitamin D.

i) *Intestinal absorption* of calcium and phosphorus is stimulated by vitamin D.

ii) *On bones.* Vitamin D is normally required for mineralisation of epiphyseal cartilage and osteoid matrix. However, in hypocalcaemia, vitamin D collaborates with parathyroid hormone and causes osteoclastic resorption of calcium and phosphorus from bone so as to maintain the normal blood levels of calcium and phosphorus.

iii) *On kidneys.* Vitamin D stimulates reabsorption of calcium at distal renal tubular level, though this function is also parathyroid hormone-dependent.

2. Immune regulation. Recently, vitamin D has been found to play an immune regulatory role due to the presence of receptors for a metabolite of vitamin D on activated lymphocytes and macrophages.

LESIONS IN VITAMIN D DEFICIENCY. Deficiency of vitamin D may result from:

- i) reduced endogenous synthesis due to inadequate exposure to sunlight;
- ii) dietary deficiency of vitamin D;
- iii) malabsorption of lipids due to lack of bile salts such as in intrahepatic biliary obstruction, pancreatic insufficiency and malabsorption syndrome;
- iv) derangements of vitamin D metabolism as occur in kidney disorders (chronic renal failure, nephrotic syndrome, uraemia), liver disorders (diffuse liver disease) and genetic disorders; and
- v) resistance of end-organ to respond to vitamin D.

Deficiency of vitamin D from any of the above mechanisms results in:

1. rickets in growing children;
2. osteomalacia in adults; and
3. hypocalcaemic tetany due to neuromuscular dysfunction.

RICKETS. The primary defects in rickets are:

- interference with mineralisation of bone; and
- deranged endochondral and intramembranous bone growth.

The pathogenesis of lesions in rickets are better understood by contrasting them with sequence of changes in normal bone growth as outlined in Table 9.5.

Rickets occurs in growing children from 6 months to 2 years of age. The disease has the following lesions and clinical characteristics (Fig. 9.11):

Skeletal changes. These are as under:

i) *Craniotables* is the earliest bony lesion occurring due to small round unossified areas in the membranous bones of the skull, disappearing within 12 months of birth. The skull looks square and box-like.

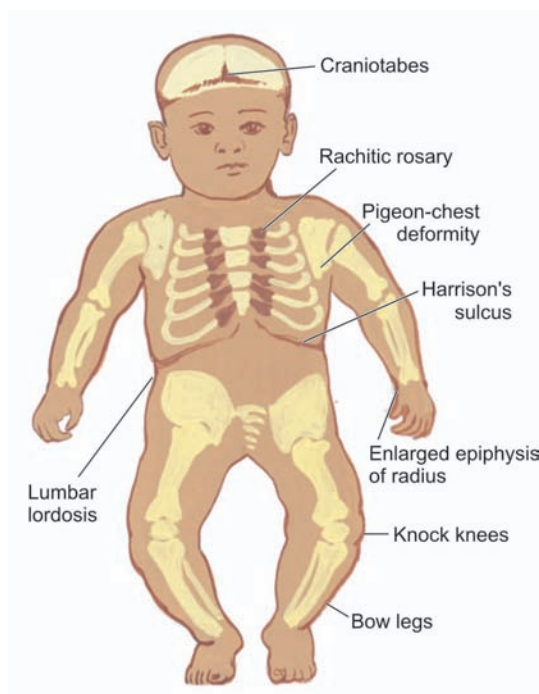


FIGURE 9.11

Lesions in rickets.

- ii) *Harrison's sulcus* appears due to indrawing of soft ribs on inspiration.
- iii) *Rachitic rosary* is a deformity of chest due to cartilaginous overgrowth at costochondral junction.
- iv) *Pigeon-chest deformity* is the anterior protrusion of sternum due to action of respiratory muscles.

v) *Bow legs* occur in ambulatory children due to weak bones of lower legs.

vi) *Knock knees* may occur due to enlarged ends of the femur, tibia and fibula.

vii) *Lower epiphyses of radius may be enlarged*.

viii) *Lumbar lordosis* is due to involvement of the spine and pelvis.

Biochemical changes. These are as follows:

- i) Lowered levels of active metabolites of vitamin D (25-hydroxy vitamin D and 1, 25-dihydroxy vitamin D).
- ii) Plasma calcium levels are normal or slightly low.
- iii) Plasma phosphate levels are lowered.
- iv) Plasma alkaline phosphatase is usually raised due to osteoblastic activity.

Vitamin D-dependent rickets is an autosomal dominant disorder of vitamin D. The disease responds rapidly to administration of 1,25-dihydroxy vitamin D.

OSTEOMALACIA. Osteomalacia is the adult counterpart of rickets in which there is failure of mineralisation of the osteoid matrix. It may occur following dietary deficiency, poor endogenous synthesis of vitamin D, or as a result of conditioned deficiency.

PATHOLOGIC CHANGES. Due to deficiency of vitamin D, osteoid matrix laid down fails to get mineralised. In H and E stained microscopic sections, this is identified by widened and thickened osteoid seams (stained pink) and decreased mineralisation at the borders between osteoid and bone (stained basophilic). *von Kossa's stain* for calcium may be employed to mark out the wide seams of unstained

TABLE 9.5: Contrasting Features of Rickets with Normal Bone Growth.

NORMAL BONE GROWTH	RICKETS
I. ENDOCHONDRAL OSSIFICATION (occurring in long tubular bones)	
i. Proliferation of cartilage cells at the epiphyses followed by provisional mineralisation	i. Proliferation of cartilage cells at the epiphyses followed by inadequate provisional mineralisation
ii. Cartilage resorption and replacement by osteoid matrix	ii. Persistence and overgrowth of epiphyseal cartilage; deposition of osteoid matrix on inadequately mineralised cartilage resulting in enlarged and expanded costochondral junctions
iii. Mineralisation to form bone	iii. Deformed bones due to lack of structural rigidity
iv. Normal vascularisation of bone	iv. Irregular overgrowth of small blood vessels in disorganised and weak bone
II. INTRAMEMBRANOUS OSSIFICATION (occurring in flat bones)	
Mesenchymal cells differentiate into osteoblasts which develop osteoid matrix and subsequent mineralisation	Mesenchymal cells differentiate into osteoblasts with laying down of osteoid matrix which fails to get mineralised resulting in soft and weak flat bones

osteoid while the calcified bone is stained black. In addition, there may be increased osteoclastic activity and fibrosis of marrow.

Clinical features. Osteomalacia is characterised by:

- i) muscular weakness;
- ii) vague bony pains;
- iii) fractures following trivial trauma;
- iv) incomplete or green-stick fractures; and
- v) looser's zones or pseudofractures at weak places in bones.

Biochemical changes. These are:

- i) normal or low serum calcium levels;
- ii) plasma phosphate levels lowered; and
- iii) raised serum alkaline phosphatase due to increased osteoblastic activity.

It may be worthwhile to note here that another chronic disorder of skeleton seen in elderly, *osteoporosis*, is clinically similar but biochemically different disease (Chapter 32).

HYPERVITAMINOSIS D. Very large excess of vitamin D may cause increased intestinal absorption of calcium and phosphorus, leading to hypercalcaemia, hyperphosphataemia and increased bone resorption. These changes may result in the following effects:

- i) increased urinary excretion of calcium and phosphate;
- ii) predisposition to renal calculi;
- iii) osteoporosis; and
- iv) widespread metastatic calcification, more marked in the renal tubules, arteries, myocardium, lungs and stomach.

Vitamin E (α -Tocopherol)

PHYSIOLOGY. Out of many naturally-occurring tocoferols, α -tocopherol is biologically the most active fat soluble compound. Vitamin E is found in most of the ordinary foods such as vegetables, grains, nuts and oils. It is absorbed from the intestine and transported in blood in the form of chylomicrons. It is stored in fat depots, liver and muscle.

The main physiologic function of vitamin E is its anti-oxidant activity. Vitamin E prevents the oxidative degradation of cell membranes containing phospholipids by its anti-oxidant property and scavenges free radicals formed by redox reaction in the body and thus maintains the integrity of the cell.

LESIONS IN VITAMIN E DEFICIENCY. The deficiency of vitamin E is mainly by conditioning disorders affecting its absorption and transport such as abetali-

poproteinaemia, intra-and extrahepatic biliary cholestasis, cystic fibrosis of pancreas and malabsorption syndrome. Low birth weight neonates, due to physiologic immaturity of liver and bowel, may also develop vitamin E deficiency. Lesions of vitamin E deficiency are as follows:

1. *Neurons* with long axons develop degeneration in the posterior columns of spinal cord.
2. *Peripheral nerves* may also develop myelin degeneration in the axons.
3. *Skeletal muscles* may develop denervation.
4. *Retinal pigmentary degeneration* may occur.
5. *Red blood cells* deficient in vitamin E such as in premature infants have reduced life span.
6. In experimental animals, vitamin E deficiency can produce *sterility* in both male and female animals.

Vitamin K

PHYSIOLOGY. Vitamin K (*K* for *Koagulations* in Danish) exists in nature in 2 forms:

- Vitamin K₁ or *phylloquinone*, obtained from exogenous dietary sources such as most green leafy vegetables; and
- Vitamin K₂ or *menadione*, produced endogenously by normal intestinal flora.

Like other fat-soluble vitamins, vitamin K is absorbed from the small intestine and requires adequate bile flow and intact pancreatic function.

The main physiologic function of vitamin K is in hepatic microsomal carboxylation reaction for vitamin K-dependent coagulation factors (most importantly factor II or prothrombin; others are factors VII, IX and X).

LESIONS IN VITAMIN K DEFICIENCY. Since vitamin K is necessary for the manufacture of prothrombin, its deficiency leads of *hypoprothrombinaemia* (Chapter 35). Estimation of plasma prothrombin, thus, affords a simple *in vitro* test for determining whether there is deficiency of vitamin K. Subjects with levels below 70% of normal should receive therapy with vitamin K.

Because most of the green vegetables contain vitamin K and that it can be synthesised endogenously, vitamin K deficiency is frequently a conditioned deficiency. The conditions which may bring about vitamin K deficiency are as follows:

1. **Haemorrhagic disease of newborn.** The newborn infants are deficient in vitamin K because of minimal stores of vitamin K at birth, lack of established intestinal flora for endogenous synthesis and limited dietary intake since breast milk is a poor source of vitamin K. Hence the clinical practice is to routinely administer vitamin K at birth.

2. Biliary obstruction. Bile is prevented from entering the bowel due to biliary obstruction so that this fat-soluble vitamin cannot be absorbed. Surgery on jaundiced patients, therefore, leads to marked tendency to bleeding.

3. Malabsorption syndrome. Patients suffering from malabsorption of fat develop vitamin K deficiency e.g. coeliac disease, sprue, pancreatic disease, hypermotility of bowel etc.

4. Anticoagulant therapy. Patients on warfarin group of anticoagulants have impaired biosynthesis of vitamin K-dependent coagulation factors.

5. Antibiotic therapy. The use of broad spectrum antibiotics and sulfa drugs reduces the normal intestinal flora.

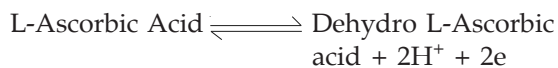
6. Diffuse liver disease. Patients with diffuse liver disease (e.g. cirrhosis, amyloidosis of liver, hepatocellular carcinoma, hepatoblastoma) have hypoprothrombinaemia due to impaired synthesis of prothrombin. Administration of vitamin K to such patients is of no avail since liver, where prothrombin synthesis utilising vitamin K takes place, is diseased.

WATER-SOLUBLE VITAMINS

Vitamin C (Ascorbic Acid)

PHYSIOLOGY. Vitamin C exists in natural sources as L-ascorbic acid closely related to glucose. The major sources of vitamin C are citrus fruits such as orange, lemon, grape fruit and some fresh vegetables like tomatoes and potatoes. It is present in small amounts in meat and milk. The vitamin is easily destroyed by heating so that boiled or pasteurised milk may lack vitamin C. It is readily absorbed from the small intestine and is stored in many tissues, most abundantly in adrenal cortex.

The **physiologic functions** of vitamin C are due to its ability to carry out *oxidation-reduction reactions*:



1. Ascorbic acid is required for hydroxylation of proline to form hydroxyproline which is an essential component of *collagen*.

2. Besides collagen, it is necessary for the *ground substance* of other mesenchymal structures such as osteoid, chondroitin sulfate, dentin and cement substance of vascular endothelium.

3. Vitamin C being a *reducing substance* has other functions such as:

- hydroxylation of dopamine to norepinephrine;

- maintenance of folic acid levels by preventing oxidation of tetrahydrofolate; and

- role in iron metabolism in its absorption, storage and keeping it in reduced state.

4. Recently, vitamin C has been found to play a role in synthesis of neurotransmitters, neuropeptide hormone synthesis and in immune response.

LESIONS IN VITAMIN C DEFICIENCY. Vitamin C deficiency in the food or as a conditioned deficiency results in scurvy. The lesions and clinical manifestations of scurvy are seen more commonly at two peak ages: in early childhood and in the very aged. These are as under (Fig. 9.12):

1. Haemorrhagic diathesis. A marked tendency to bleeding is characteristic of scurvy. This may be due to deficiency of intercellular cement which holds together the cells of capillary endothelium. There may be haemorrhages in the skin, mucous membranes, gums, muscles, joints and underneath the periosteum.

2. Skeletal lesions. These changes are more pronounced in growing children. The most prominent change is the *deranged formation of osteoid matrix and not deranged mineralisation* (c.f. the pathological changes underlying rickets already described). Growing tubular bones as well as flat bones are affected. The epiphyseal

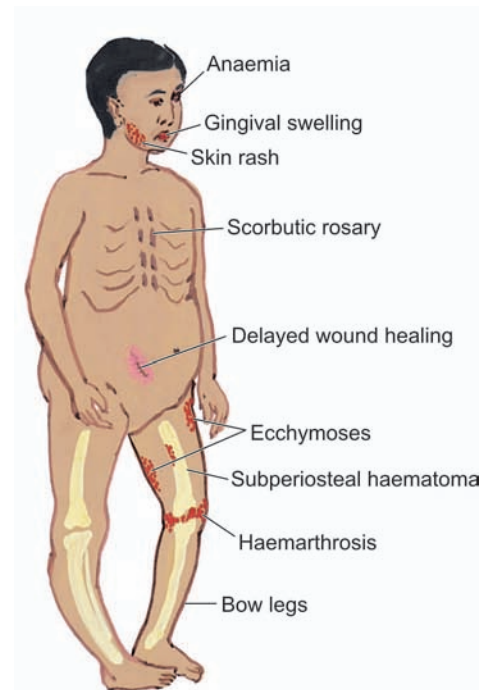


FIGURE 9.12
Lesions in scurvy.

ends of growing long bones have cartilage cells in rows which normally undergo provisional mineralisation. However, due to vitamin C deficiency, the next step of laying down of osteoid matrix by osteoblasts is poor and results in failure of resorption of cartilage. Consequently, mineralised cartilage under the widened and irregular epiphyseal plates project as *scorbutic rosary*. The skeletal changes are further worsened due to haemorrhages and haematomas under the periosteum and bleeding into the joint spaces.

3. Delayed wound healing. There is delayed healing of wounds in scurvy due to:

- deranged collagen synthesis;
- poor preservation and maturation of fibroblasts; and
- localisation of infections in the wounds.

4. Anaemia. Anaemia is common in scurvy. It may be the result of haemorrhage, interference with formation of folic acid or deranged iron metabolism. Accordingly, anaemia is most often normocytic normochromic type; occasionally it may be megaloblastic or even iron deficiency type.

5. Lesions in teeth and gums. Scurvy may interfere with development of dentin. The gums are soft and swollen, may bleed readily and get infected commonly.

6. Skin rash. Hyperkeratotic and follicular rash may occur in scurvy.

VITAMIN B COMPLEX

The term vitamin B was originally coined for a substance capable of curing beriberi (B from beriberi). Now, vitamin B complex is commonly used for a *group of essential compounds which are biochemically unrelated but occur together in certain foods* such as green leafy vegetables, cereals, yeast, liver and milk. Most of the vitamins in this group are involved in metabolism of proteins, carbohydrates and fats.

The principal members of vitamin B complex are thiamine (vitamin B₁), riboflavin (vitamin B₂), niacin (nicotinic acid), pyridoxine (vitamin B₆), folate (folic acid) and cyanocobalamin (vitamin B₁₂). Two other compounds—biotin and pantothenic acid, are also considered components of vitamin B complex but there is no definite evidence that any clinical disorder results from their deficiency.

Thiamine (Vitamin B₁)

PHYSIOLOGY. Thiamine hydrochloride is available in a variety of items of diet such as peas, beans, pulses, yeast, green vegetable roots, fruits, meat, pork, rice and wheat bran. The vitamin is lost in refined foods such as

polished rice, white flour and white sugar. A few substances in the diet (strong tea, coffee) act as *anti-thiamines*. Since the vitamin is soluble in water, considerable amount of the vitamin is lost during cooking of vegetables. The vitamin is absorbed from the intestine either by passive diffusion or by energy-dependent transport. Reserves of vitamin B₁ are stored in skeletal muscles, heart, liver, kidneys and bones.

The main **physiologic function** of thiamine is in carbohydrate metabolism. Thiamine after absorption is phosphorylated to form thiamine pyrophosphate which is the functionally active compound. This compound acts as coenzyme for carboxylase so as to decarboxylate pyruvic acid, synthesises ATP and also participates in the synthesis of fat from carbohydrate.

LESIONS IN THIAMINE DEFICIENCY. Thiamine deficiency, primary or conditioned, leads to failure of complete combustion of carbohydrate and accumulation of pyruvic acid. This results in beriberi which produces lesions at 3 target tissues (peripheral nerves, heart and brain). Accordingly, beriberi is of 3 types:

- dry beriberi (*peripheral neuritis*);
- wet beriberi (*cardiac manifestations*), and
- cerebral beriberi (*Wernicke-Korsakoff's syndrome*).

It is worth-noting that lesions in beriberi are mainly located in the nervous system and heart. This is because the energy requirement of the brain and nerves is solely derived from oxidation of carbohydrates which is deranged in beriberi. Lesions in the heart appear to arise due to reduced ATP synthesis in beriberi which is required for cardiac functions.

The features of 3 forms of beriberi are as under:

1. Dry beriberi (peripheral neuritis). This is marked by neuromuscular symptoms such as weakness, paraesthesia and sensory loss. The nerves show polyneuritis, myelin degeneration and fragmentation of axons.

2. Wet beriberi (cardiac manifestations). This is characterised by cardiovascular involvement, generalised oedema, serous effusions and chronic passive congestion of viscera. The heart in beriberi is flabby (due to thin and weak myocardium), enlarged and globular in appearance due to dilatation of all the 4 chambers (Fig. 9.13).

Microscopic examination of heart shows hydropic degeneration of myocardial fibres, loss of striations, interstitial oedema and lymphocytic infiltration.

3. Cerebral beriberi (Wernicke-Korsakoff's syndrome). It consists of the following features:

i) *Wernicke's encephalopathy* occurs more often due to conditioned deficiencies such as in chronic alcoholism.

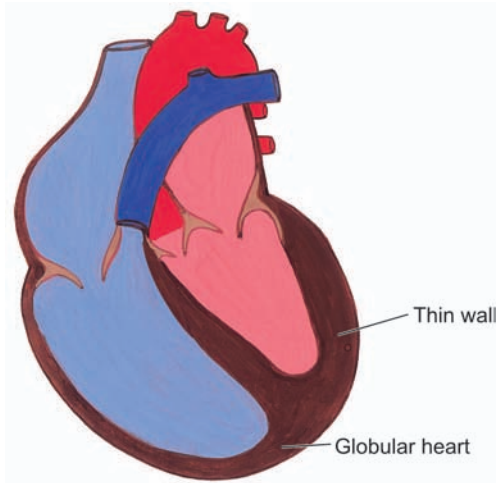


FIGURE 9.13

Wet (Cardiac) beriberi. Flabby, thin-walled, enlarged and globular appearance of the heart due to four-chamber dilatation.

It is characterised by degeneration of ganglia cells, focal demyelination and haemorrhage in the nuclei surrounding the region of ventricles and aqueduct.

Microscopic examination shows degeneration and necrosis of neurons, hypertrophy-hyperplasia of small blood vessels and haemorrhages.

ii) *Korsakoff's psychosis* results from persistence of psychotic features following brain haemorrhage in Wernicke's encephalopathy.

Riboflavin (Vitamin B₂)

PHYSIOLOGY. Riboflavin used to be called 'yellow respiratory enzyme' (*flavus* = yellow), currently known as 'cytochrome oxidase enzyme' which is important in cellular respiration. The vitamin is usually distributed in plant and animal foods such as the liver, beef, mutton, pork, eggs, milk and green vegetables. Like other water-soluble vitamins, it is rapidly absorbed from the bowel and stored in tissues like liver.

LESIONS IN RIBOFLAVIN DEFICIENCY. Lesions due to primary or conditioned deficiency of riboflavin (*ariboflavinosis*) are as follows:

1. *Ocular lesions* consist of vascularisation of normally avascular cornea due to proliferation of capillaries from limbus. Subsequently, conjunctivitis, interstitial keratitis and corneal ulcers may develop.
2. *Cheilosis* and *angular stomatitis* are characterised by occurrence of fissures and cracks at the angles of mouth.

3. *Glossitis* is development of red, cyanosed and shiny tongue due to atrophy of mucosa of tongue ('bald tongue').

4. *Skin changes* appear in the form of scaly dermatitis resembling seborrheic dermatitis on nasolabial folds on face, scrotum and vulva.

Niacin (Nicotinic Acid)

PHYSIOLOGY. As with thiamine and riboflavin, niacin is also widely distributed in plant and animal foods such as the liver, kidney, meat, green vegetables and whole grain cereals. Niacin includes biologically active derivative *nicotinamide* which is essential for the formation of 2 oxidative coenzymes (*dehydrogenases*):

■ NAD (nicotinamide adenine dinucleotide) which is required for dehydrogenation in the metabolism of fat, carbohydrates and proteins.

■ NADP (nicotinamide adenine dinucleotide phosphate) which is essential for dehydrogenation in the hexose monophosphate shunt of glucose metabolism.

LESIONS IN NIACIN DEFICIENCY. Deficiency of niacin causes pellagra, so named because of the rough skin of such patients (Italian *pelle agra* = rough skin). Pellagra may result from dietary deficiency in those who largely subsist on maize since niacin in maize is present in bound form and hence not absorbable. Since niacin can be endogenously synthesised from tryptophan, a diet deficient in this amino acid or disorders of tryptophan metabolism such as in carcinoid syndrome or Hartnup syndrome results in niacin deficiency.

Lesions in pellagra are characterised by 3D's:

1. *Dermatitis*: The sun-exposed areas of skin develop erythema resembling sunburn. This may progress to chronic type of dermatitis with blister formation.
2. *Diarrhoea*: Lesions similar to those seen in skin may develop in mucous membrane of the alimentary tract resulting in glossitis, lesions in mouth, oesophagus, stomach and colon and cause diarrhoea, nausea, vomiting and burning sensation.
3. *Dementia*: Degeneration of neurons of the brain and of spinal tract results in neurological symptoms such as dementia, peripheral neuritis, ataxia and visual and auditory disturbances.

Pyridoxine (Vitamin B₆)

PHYSIOLOGY. Pyridoxine or vitamin B₆ is widely distributed in all animal and plant foods such as meat, liver, eggs, green vegetables and whole grain cereals. Pyridoxine exists in 3 closely related naturally-occurring

substances—*pyridoxine*, *pyridoxal* and *pyridoxamine*. All of these can be converted into biologically active coenzyme, pyridoxal 5-phosphate.

The major **physiologic functions** of pyridoxine are related to:

- fat metabolism;
- protein metabolism;
- amino acid metabolism such as decarboxylation of amino acids, transmethylation of methionine, tryptophan metabolism and formation of melanin;
- transmission of neural impulses; and
- in the immune response.

LESIONS IN PYRIDOXINE DEFICIENCY. Vitamin B₆ deficiency may result from inadequate dietary intake or may result from secondary deficiency such as increased demand in pregnancy and lactation, chronic alcoholism and intake of certain drugs (e.g. isoniazid in the treatment of tuberculosis, penicillamine, oestrogen in oral contraceptives etc).

The **lesions** of pyridoxine deficiency are vague and include the following:

1. Convulsions in infants born to mothers who had been administered large doses of vitamin B₆ for hyperemesis gravidarum (pyridoxine dependence)
2. Dermatitis
3. Cheilosis and angular stomatitis
4. Glossitis (bald tongue)
5. Sideroblastic anaemia.

Folate (Folic Acid) and Cyanocobalamin (Vitamin B₁₂)

Both these vitamins included in the B complex group are required for red cell formation. Their deficiency leads to megaloblastic anaemia which is described in Chapter 33.

In closing the discussion of vitamin B complex, it must be mentioned that many of the animal and plant foods contain vitamin B complex group of vitamins. Their deficiency, whether primary from poverty, ignorance etc, or secondary from conditioning factors like chronic alcoholism, is more frequently *multiple vitamin deficiency*. Hence, the clinical practice is to administer combination of these members of vitamin B complex.

TRACE ELEMENTS

Several minerals in trace amounts are essential for health since they form components of enzymes and cofactors for metabolic functions. Besides *calcium* and *phosphorus* required for vitamin D manufacture, others include: *iron*,

copper, *iodine*, *zinc*, *selenium*, *manganese*, *nickel*, *chromium*, *molybdenum*, *fluorine*. However, out of these, the dietary deficiency of first five trace elements is associated with deficiency states which are discussed in detail in respective chapters later. These are:

- i) *Iron*: Microcytic hypochromic anaemia.
- ii) *Copper*: Muscle weakness, neurologic defect.
- iii) *Iodine*: Goitre and hyperthyroidism.
- iv) *Zinc*: Growth retardation, infertility.
- v) *Selenium*: Myopathy, cardiomyopathy.

DIET AND CANCER

Before closing the discussion of environmental and nutritional pathology, it is worthwhile to sum up its relationship to carcinogenesis. There are three possible mechanisms on which the story of this relationship can be built up:

1. Dietary content of exogenous carcinogens:

- i) The most important example in this mechanism comes from naturally-occurring carcinogen *aflatoxin* which is strongly associated with high incidence of hepatocellular carcinoma in those consuming grain contaminated with *Aspergillus flavus*.
- ii) *Artificial sweeteners* (e.g. saccharine cyclamates), food additives and pesticide contamination of food are implicated as carcinogens derived from diet.

2. Endogenous synthesis of carcinogens or promoters:

- i) In the context of etiology of *gastric carcinoma*, nitrites, nitrates and amines from the digested food are transformed in the body to carcinogens—nitrosamines and nitrosamides.
- ii) In the etiology of *colon cancer*, low fibre intake and high animal-derived fats are implicated. High fat diet results in rise in the level of bile acids and their metabolites produced by intestinal bacteria which then act as carcinogens. The low fibre diet, on the other hand, does not provide adequate protection to the mucosa and reduces the stool bulk and thus increases the time the stools remain in the colon.
- iii) In the etiology of *breast cancer*, epidemiologic studies have implicated the role of animal proteins, fats and obesity with as yet unsubstantiated evidence.

3. Inadequate protective factors: As already mentioned, some components of diet such as *vitamin C*, *A*, *E*, *selenium*, and *β-carotenes* have protective role against cancer. These substances in normal amounts in the body act as antioxidants and protect the cells against free radical injury but their role of supplementation in diet as prevention against cancer is unproven.



Inflammation and Healing, Immunity and Hypersensitivity, Infections and Infestations

SECTION CONTENTS

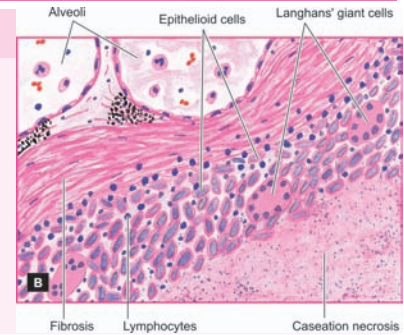
CHAPTER 10: Acute and Chronic Inflammation

CHAPTER 11: Granulomatous Inflammation

CHAPTER 12: Healing of Tissues

CHAPTER 13: Immunity and Hypersensitivity

CHAPTER 14: Infections and Infestations



10

Acute and Chronic Inflammation

DEFINITION AND CAUSES. Inflammation is defined as the local response of living mammalian tissues to injury due to any agent. It is a body defense reaction in order to eliminate or limit the spread of injurious agent as well as to remove the consequent necrosed cells and tissues.

The agents causing inflammation may be as under:

1. *Physical agents* like heat, cold, radiation, mechanical trauma.
2. *Chemical agents* like organic and inorganic poisons.
3. *Infective agents* like bacteria, viruses and their toxins.
4. *Immunological agents* like cell-mediated and antigen-antibody reactions.

Thus, *inflammation is distinct from infection*—the former being a protective response by the body while the latter is invasion into the body by harmful microbes and their resultant ill-effects by toxins. Inflammation involves 2 basic processes with some overlapping, viz. early *inflammatory response* and later followed by *healing*. Though both these processes generally have protective role against injurious agents, inflammation and healing may cause considerable harm to the body as well e.g. anaphylaxis to bites by insects or reptiles, drugs, toxins, atherosclerosis, chronic rheumatoid arthritis, fibrous bands and adhesions in intestinal obstruction.

SIGNS OF INFLAMMATION. The Roman writer Celsus in 1st century A.D. named the famous 4 *cardinal signs of inflammation* as:

- *rubor* (redness);
- *tumor* (swelling);
- *calor* (heat); and
- *dolor* (pain).

To these, fifth sign *functio laesa* (loss of function) was later added by Virchow. The word inflammation means burning. This nomenclature had its origin in old times but now we know that burning is only one of the signs of inflammation.

TYPES OF INFLAMMATION. Depending upon the defense capacity of the host and duration of response, inflammation can be classified as acute and chronic.

I. *Acute inflammation* is of short duration and represents the early body reaction and is usually followed by repair.

The main features of acute inflammation are:

1. accumulation of fluid and plasma at the affected site;
2. intravascular activation of platelets; and
3. polymorphonuclear neutrophils as inflammatory cells.

II. *Chronic inflammation* is of longer duration and occurs either after the causative agent of acute inflammation

persists for a long time, or the stimulus is such that it induces chronic inflammation from the beginning.

The characteristic feature of chronic inflammation is presence of chronic inflammatory cells such as lymphocytes, plasma cells and macrophages.

ACUTE INFLAMMATION

The changes in acute inflammation can be conveniently described under the following 2 headings:

- I. Vascular events.
- II. Cellular events.

I. VASCULAR EVENTS

Alteration in the microvasculature (arterioles, capillaries and venules) is the earliest response to tissue injury. These alterations include: haemodynamic changes and changes in vascular permeability.

Haemodynamic Changes

The earliest features of inflammatory response result from changes in the vascular flow and calibre of small blood vessels in the injured tissue. The sequence of these changes is as under:

1. Irrespective of the type of injury, immediate vascular response is of **transient vasoconstriction** of arterioles. With mild form of injury, the blood flow may be re-established in 3-5 seconds while with more severe injury the vasoconstriction may last for about 5 minutes.
2. Next follows **persistent progressive vasodilatation** which involves mainly the arterioles, but to a lesser extent, affects other components of the microcirculation

like venules and capillaries. This change is obvious within half an hour of injury. Vasodilatation results in increased blood volume in microvascular bed of the area, which is responsible for redness and warmth at the site of acute inflammation.

3. Progressive vasodilatation, in turn, may elevate the **local hydrostatic pressure** resulting in transudation of fluid into the extracellular space. This is responsible for swelling at the local site of acute inflammation.

4. **Slowing or stasis** of microcirculation occurs next. Slowing is attributed to increased permeability of microvasculature that results in increased concentration of red cells, and thus, raised blood viscosity.

5. Stasis or slowing is followed by **leucocytic margination** or peripheral orientation of leucocytes (mainly neutrophils) along the vascular endothelium. The leucocytes stick to the vascular endothelium briefly, and then move and migrate through the gaps between the endothelial cells into the extravascular space. This process is known as *emigration* (discussed later in detail).

The features of haemodynamic changes in inflammation are best demonstrated by the **Lewis experiment**. Lewis induced the changes in the skin of inner aspect of forearm by firm stroking with a blunt point. The reaction so elicited is known as *triple response* or *red line response* consisting of the following (Fig. 10.1):

- i) *Red line* appears within a few seconds following stroking and results from local vasodilatation of capillaries and venules.
- ii) *Flare* is the bright reddish appearance or flush surrounding the red line and results from vasodilatation of the adjacent arterioles.

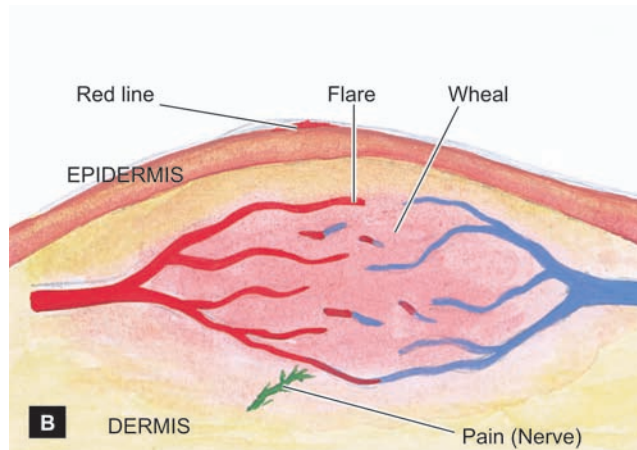
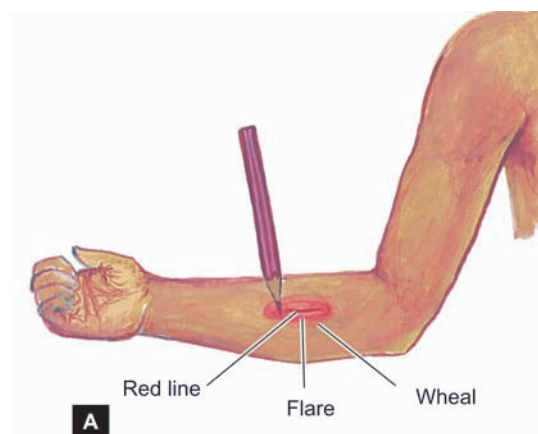


FIGURE 10.1

A, 'Triple response' elicited by firm stroking of skin of forearm with a pencil. B, Diagrammatic view of microscopic features of triple response of the skin.

iii) *Wheal* is the swelling or oedema of the surrounding skin occurring due to transudation of fluid into the extravascular space.

These features, thus, elicit the classical signs of inflammation—redness, heat, swelling and pain.

Altered Vascular Permeability

PATHOGENESIS. In and around the inflamed tissue, there is accumulation of oedema fluid in the interstitial compartment which comes from blood plasma by its escape through the endothelial wall of peripheral vascular bed. In the initial stage, the escape of fluid is due to vasodilatation and consequent elevation in hydrostatic pressure. This is transudate in nature. But subsequently, the characteristic inflammatory oedema, exudate, appears by increased vascular permeability of microcirculation. The differences between transudate and exudate, are summarised on page 189.

The appearance of inflammatory oedema due to increased vascular permeability of microvascular bed is explained on the basis of **Starling's hypothesis**. In

normal circumstances, the fluid balance is maintained by two opposing sets of forces:

- Forces that cause **outward movement** of fluid from microcirculation are *intravascular hydrostatic pressure* and *osmotic pressure of interstitial fluid*.
- Forces that cause **inward movement** of interstitial fluid into circulation are *intravascular osmotic pressure* and *hydrostatic pressure of interstitial fluid*.

Whatever little fluid is left in the interstitial compartment is drained away by lymphatics and, thus, no oedema results normally (Fig. 10.2,A). However, in inflamed tissues, the endothelial lining of microvasculature becomes more leaky. Consequently, intravascular osmotic pressure decreases and osmotic pressure of the interstitial fluid increases resulting in excessive outward flow of fluid into the interstitial compartment which is exudative inflammatory oedema (Fig. 10.2,B).

MECHANISMS OF INCREASED VASCULAR PERMEABILITY. In acute inflammation, normally non-permeable endothelial layer of microvasculature becomes leaky. This is explained by one or more of the

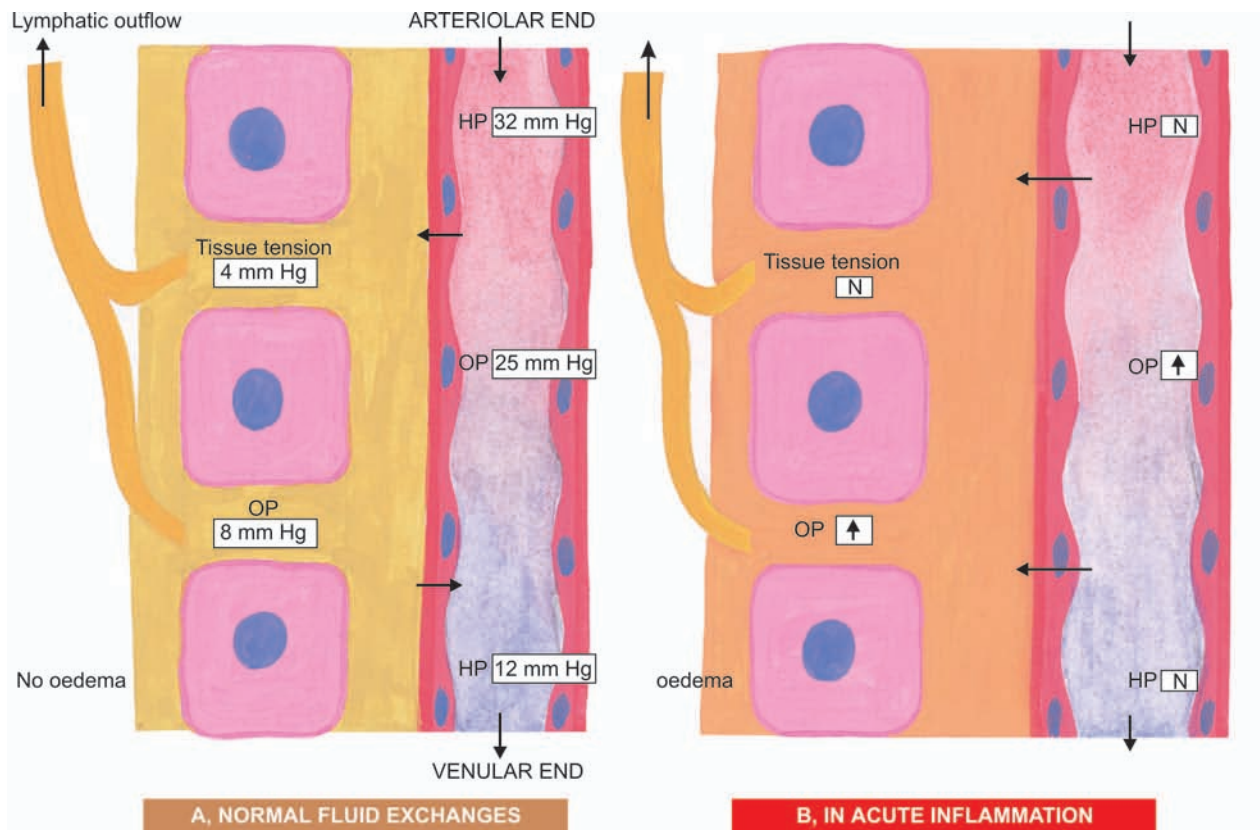


FIGURE 10.2

Fluid interchange between blood and extracellular fluid (ECF). (HP = hydrostatic pressure, OP = osmotic pressure).

following mechanisms which are diagrammatically illustrated in Fig. 10.3.

i) Contraction of endothelial cells. This is the most common mechanism of increased leakiness that affects venules exclusively while capillaries and arterioles remain unaffected. The endothelial cells develop temporary gaps between them due to their contraction resulting in vascular leakiness. It is mediated by the release of histamine, bradykinin and other chemical mediators. The response begins immediately after injury, is usually reversible, and is for short duration (15-30 minutes).

Example of such *immediate transient leakage* is mild thermal injury of skin of forearm.

ii) Retraction of endothelial cells. In this mechanism, there is structural re-organisation of the cytoskeleton of endothelial cells that causes reversible retraction at the intercellular junctions. This change too affects venules and is mediated by cytokines such as interleukin-1 (IL-1) and tumour necrosis factor (TNF). The onset of response takes 4-6 hours after injury and lasts for 24 hours or more (*somewhat delayed and prolonged leakage*).

The example of this type of response exists *in vitro* experimental work only.

iii) Direct injury to endothelial cells. Direct injury to the endothelium causes cell necrosis and appearance of physical gaps at the sites of detached endothelial cells. Process of thrombosis is initiated at the site of damaged endothelial cells. The change affects all levels of microvasculature (venules, capillaries and arterioles). The increased permeability may either appear imme-

diately after injury and last for several hours or days (*immediate sustained leakage*), or may occur after a delay of 2-12 hours and last for hours or days (*delayed prolonged leakage*).

The examples of immediate sustained leakage are severe bacterial infections while delayed prolonged leakage may occur following moderate thermal injury and radiation injury.

iv) Endothelial injury mediated by leucocytes. Adherence of leucocytes to the endothelium at the site of inflammation may result in activation of leucocytes. The activated leucocytes release proteolytic enzymes and toxic oxygen species which may cause endothelial injury and increased vascular leakiness. This form of increased vascular leakiness affects mostly venules and is a *late response*.

The examples are seen in sites where leucocytes adhere to the vascular endothelium e.g. in pulmonary venules and capillaries.

v) Neovascularisation. In addition, the newly formed capillaries under the influence of vascular endothelial growth factor (VEGF) during the process of repair and in tumours are excessively leaky.

These mechanisms are summarised in Table 10.1.

II. CELLULAR EVENTS

The cellular phase of inflammation consists of 2 processes:

1. exudation of leucocytes; and
2. phagocytosis.

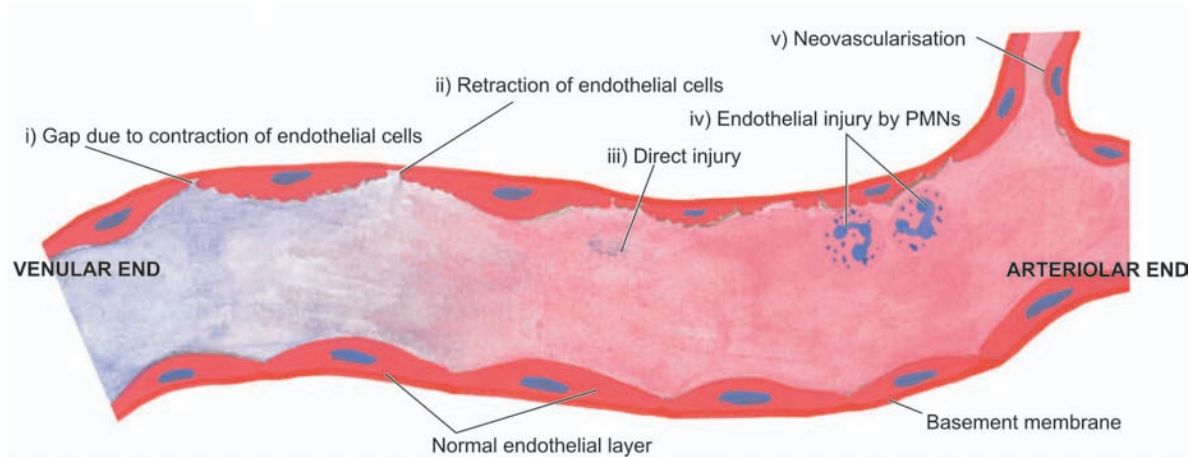


FIGURE 10.3

Schematic illustration of pathogenesis of increased vascular permeability in acute inflammation. The serial numbers in the figure correspond to 5 numbers in the text.

TABLE 10.1: Mechanisms of Increased Vascular Permeability.

MECHANISM	MICROVASCULATURE	RESPONSE TYPE	PATHOGENESIS	EXAMPLES
1. <i>Endothelial cell contraction</i>	Venules	Immediate transient (15-30 min)	Histamine, bradykinin, others	Mild thermal injury
2. <i>Endothelial cell retraction</i>	Venules	Somewhat delayed (in 4-6 hrs) prolonged (for 24 hrs or more)	IL-1, TNF	<i>In vitro</i> only
3. <i>Direct endothelial cell injury</i>	Arterioles, venules, capillaries	Immediate prolonged (hrs to days), or delayed (2-12 hrs) prolonged (hrs to days)	Cell necrosis and detachment	Moderate to severe burns, severe bacterial infection, radiation injury
4. <i>Leucocyte-mediated endothelial injury</i>	Venules, capillaries	Delayed, prolonged	Leucocyte activation	Pulmonary venules and capillaries
5. <i>Neovascularisation</i>	All levels	Any type	Angiogenesis, VEGF	Healing, tumours

Exudation of Leucocytes

The escape of leucocytes from the lumen of microvasculature to the interstitial tissue is the most important feature of inflammatory response. In acute inflammation, polymorphonuclear neutrophils (PMNs) comprise the first line of body defense, followed later by monocytes and macrophages.

The changes leading to migration of leucocytes are as follows:

1. CHANGES IN THE FORMED ELEMENTS OF BLOOD. In the early stage of inflammation, the rate of flow of blood is increased due to vasodilatation. But subsequently, there is slowing or stasis of bloodstream. With stasis, changes in the normal axial flow of blood in the microcirculation take place. The normal axial flow consists of central stream of cells comprised by leucocytes and RBCs and peripheral cell-free layer of plasma close to vessel wall (Fig. 10.4,A). Due to slowing and stasis, the central stream of cells widens and peripheral plasma zone becomes narrower because of loss of plasma by exudation. This phenomenon is known as *margination*. As a result of this redistribution, the neutrophils of the central column come close to the vessel wall; this is known as *pavementing* (Fig. 10.4,B).

2. ROLLING AND ADHESION. Peripherally margined and paved neutrophils slowly roll over the endothelial cells lining the vessel wall (*rolling phase*) (Fig. 10.4,C). This is followed by the transient bond between the leucocytes and endothelial cells becoming firmer (*adhesion phase*). The following adhesion molecules bring about rolling and adhesion phases:

i) **Selectins** mediate rolling of PMNs over endothelial surface. These consist of *P-selectin* (preformed and stored

in endothelial cells and platelets), *E-selectin* (synthesised by cytokine-activated endothelial cells) and *L-selectin* (expressed on the surface of lymphocytes and neutrophils).

ii) **Integrins** on the endothelial cell surface are activated during the process of loose and transient adhesions between endothelial cells and leucocytes. At the same time the receptors for integrins on the neutrophils are also stimulated. This process brings about firm adhesion between leucocyte and endothelium.

iii) **Immunoglobulin superfamily adhesion molecule** such as intercellular adhesion molecule (ICAM-1, 2) help in localising leucocytes to the site of tissue injury and thus help in transmigration of PMNs.

3. EMIGRATION. After sticking of neutrophils to endothelium, the former move along the endothelial surface till a suitable site between the endothelial cells is found where the neutrophils throw out cytoplasmic pseudopods. Subsequently, the neutrophils lodged between the endothelial cells and basement membrane cross the basement membrane by damaging it locally with secreted collagenases and escape out into the extravascular space; this is known as *emigration* (Fig. 10.4,D). The damaged basement membrane is repaired almost immediately. As already mentioned, neutrophils are the dominant cells in acute inflammatory exudate in the first 24 hours, and monocyte-macrophages appear in the next 24-48 hours. However, neutrophils are short-lived (24-48 hours) while monocyte-macrophages survive much longer.

Simultaneous to emigration of leucocytes, escape of red cells through gaps between the endothelial cells, *diapedesis*, takes place. It is a passive phenomenon—RBCs being forced out either by raised hydrostatic pressure or may escape through the endothelial defects

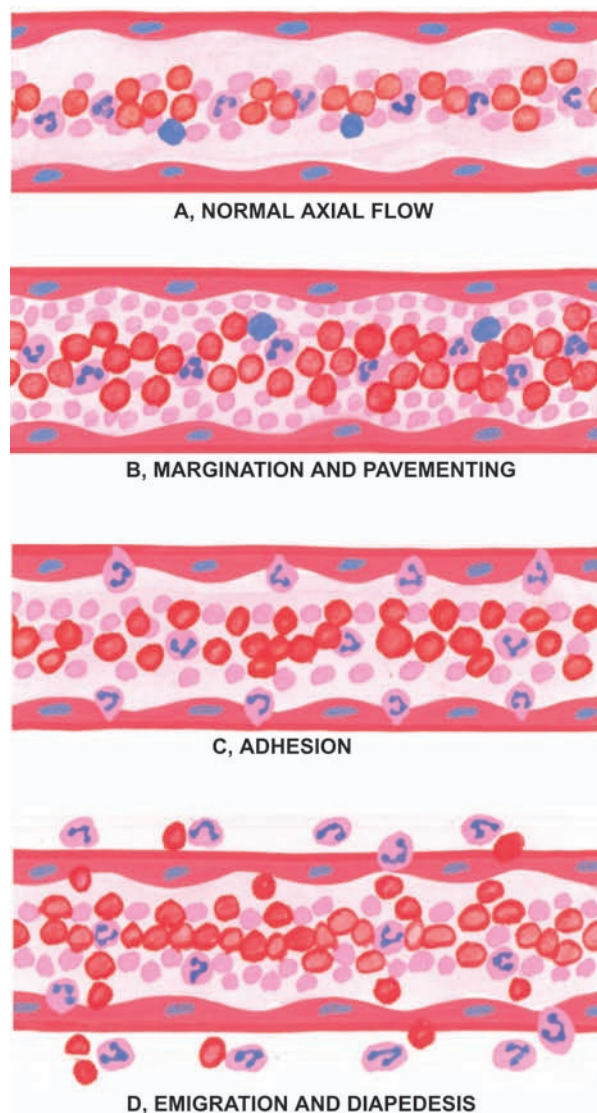


FIGURE 10.4

Sequence of changes in the exudation of leucocytes. A, Normal axial flow of blood with central column of cells and peripheral zone of cell-free plasma. B, Margination and pavementing of neutrophils with narrow plasmatic zone. C, Adhesion of neutrophils to endothelial cells with pseudopods in the intercellular junctions. D, Emigration of neutrophils and diapedesis with damaged basement membrane.

left after emigration of leucocytes. Diapedesis gives haemorrhagic appearance to the inflammatory exudate.

4. CHEMOTAXIS. The chemotactic factor-mediated transmigration of leucocytes after crossing several barriers (endothelium, basement membrane, perivascular myofibroblasts and matrix) to reach the interstitial tissues is called chemotaxis. The concept of chemotaxis is well illustrated by *Boyden's chamber*

experiment. In this, a millipore filter (3 μm pore size) separates the suspension of leucocytes from the test solution in tissue culture chamber. If the test solution contains chemotactic agent, the leucocytes migrate through the pores of filter towards the chemotactic agent (Fig. 10.5).

The agents acting as potent chemotactic substances for different leucocytes called *chemokines* are as follows:

- i) Leukotriene B_4 (LT- B_4)
- ii) Platelet factor 4 (PF $_4$)
- iii) Components of complement system (C $_3$, C $_5$ in particular)
- iv) Cytokines (Interleukins IL-1, IL-5, IL-6)
- v) Soluble bacterial products (such as formylated peptides)
- vi) Monocyte chemoattractant protein (MCP-1)
- vii) Chemotactic factor for CD $_4$ +T cells
- viii) Eotaxin chemotactic for eosinophils.

There are specific receptors for each of the chemoattractants listed above. In addition, chemotactic agents also induce leucocyte activation that includes: the production of arachidonic acid metabolites, degranulation and secretion of lysosomal enzymes, generation of oxygen metabolites, increased intracellular calcium, and increase in leucocyte surface adhesion molecules.

Phagocytosis

Phagocytosis is defined as the process of engulfment of solid particulate material by the cells (cell-eating). The cells performing this function are called *phagocytes*. There are 2 main types of phagocytic cells:

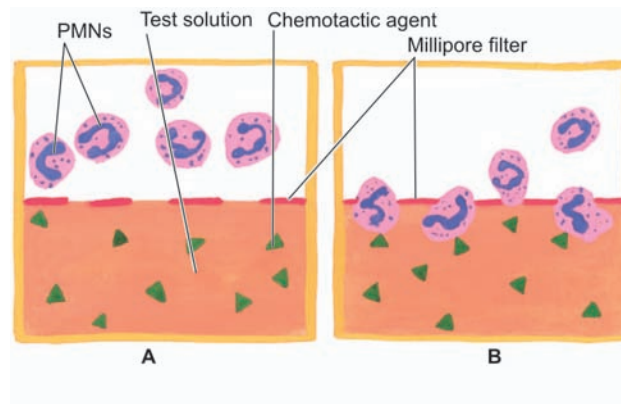


FIGURE 10.5

The Boyden's chamber with millipore filter, shown by dotted line. A, Suspension of leucocytes above is separated from test solution below. B, Lower half of chamber shows migration of neutrophils towards chemotactic agent.

i) Polymorphonuclear neutrophils (PMNs) which appear early in acute inflammatory response, also called as *microphages*.

ii) Circulating monocytes and fixed tissue mononuclear phagocytes called as *macrophages*.

The process of phagocytosis is similar for both polymorphs and macrophages and involves the following 4 steps:

1. Recognition and attachment stage (opsonisation)
2. Engulfment stage
3. Secretion (degranulation) stage
4. Digestion or degradation stage.

1. RECOGNITION AND ATTACHMENT STAGE.

The phagocytic cells are recognised and attracted to bacteria by chemotactic factors released by bacterial products as well as by tissue proteins. In order to establish a bond between bacteria and the cell membrane of phagocytic cell, the micro-organisms get coated with *opsonins* which are naturally-occurring factors in the serum. The main opsonins present in the serum and their corresponding receptors on the surface of phagocytic cells (PMNs or macrophages) are as under (Fig. 10.6,A):

- i) *IgG opsonin* is the Fc fragment of immunoglobulin G; it is the naturally occurring antibody in the serum that coats the bacteria while the PMNs possess receptors for the same.
- ii) *C_{3b} opsonin* is the fragment of complement; it is generated by activation of complement pathway. It is strongly chemotactic for attracting PMNs to bacteria.
- iii) *Lectins* are carbohydrate-binding proteins in the plasma which bind to bacterial cell wall.

2. ENGULFMENT STAGE. The opsonised particle bound to the surface of phagocyte is ready to be engulfed. This is accomplished by formation of cytoplasmic pseudopods around the particle due to activation of actin filaments beneath the cell wall, enveloping it in a phagocytic vacuole (Fig. 10.6,B). Eventually, the plasma membrane enclosing the phagocytic vacuole breaks from the cell surface so that membrane lined phagocytic vacuole lies free in the cell cytoplasm (Fig. 10.6,C). The lysosomes of the cell fuse with the phagocytic vacuole and form phagolysosome or phagosome (Fig. 10.6,D).

3. DEGRANULATION STAGE. During this process, the preformed granule-stored products of PMNs are discharged or secreted into the phagosome and the extracellular environment. In particular, the specific or secondary granules of PMNs are discharged (e.g. lysosomes) while the azurophilic granules are fused with phagosomes. Besides the discharge of preformed granules, mononuclear phagocytes synthesise and secrete certain enzymes (e.g. interleukin 2 and 6, TNF), arachidonic acid metabolites (e.g. prostaglandins, leukotrienes, platelet activating factor) and oxygen metabolites (e.g. superoxide oxygen, hydrogen peroxide, hypochlorous acid).

4. KILLING OR DEGRADATION STAGE. Next comes the stage of killing and digestion of micro-organism completing the role of phagocytes as scavenger cells. The micro-organisms after being killed by antibacterial substances are degraded by hydrolytic enzymes. However, this mechanism fails to kill and degrade some bacteria like tubercle bacilli.

The antimicrobial agents act by either of the following mechanisms:

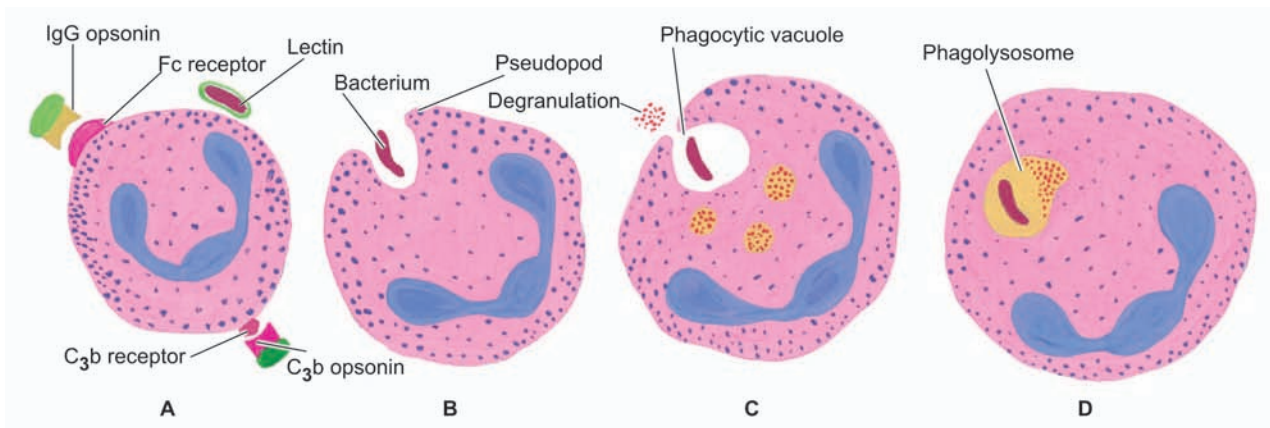


FIGURE 10.6

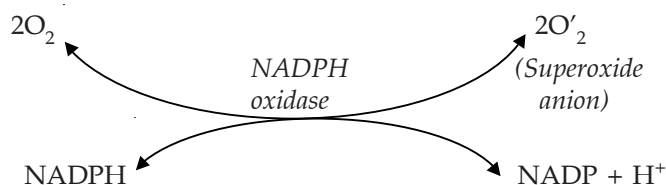
Stages in phagocytosis of a foreign particle. A, Opsonisation of the particle. B, Pseudopod engulfing the opsonised particle. C, Incorporation within the cell (phagocytic vacuole) and degranulation. D, Phagolysosome formation after fusion of lysosome of the cell.

- i) Oxygen-dependent bactericidal mechanism;
- ii) Oxygen-independent bactericidal mechanism; and
- iii) Nitric oxide mechanism.

i) Oxygen-dependent bactericidal mechanism. An important mechanism of microbicidal killing is by the production of reactive oxygen metabolites (O'_2 , H_2O_2 , $OH^\cdot$, $HOCl$, HOI , $HOBr$).

A phase of increased oxygen consumption ('respiratory burst') by activated phagocytic leucocytes requires the essential presence of NADPH oxidase.

NADPH-oxidase present in the cell membrane of phagosome reduces oxygen to superoxide ion (O'_2):



Superoxide is subsequently converted into H_2O_2 which has bactericidal properties:

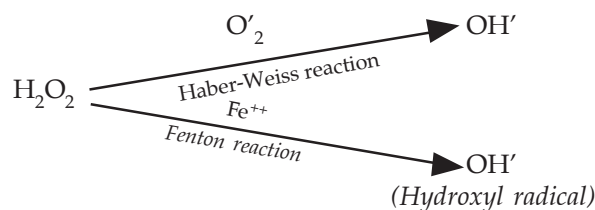


This type of bactericidal activity is carried out either via enzyme myeloperoxidase (MPO) present in the granules of neutrophils and monocytes, or independent of enzyme MPO, as under:

a) **MPO-dependent killing (H_2O_2 -MPO-halide system).** In this mechanism, the enzyme MPO acts on H_2O_2 in the presence of halides (chloride, iodide or bromide) to form hypohalous acid ($HOCl$, HOI , $HOBr$) which is more potent antibacterial agent than H_2O_2 :



b) **MPO-independent killing.** Mature macrophages lack the enzyme MPO and they carry out bactericidal activity by producing $OH^\cdot$ ions and superoxide singlet oxygen (O') from H_2O_2 in the presence of O'_2 (Haber-Weiss reaction) or in the presence of Fe^{++} (Fenton reaction):



Reactive oxygen metabolites are particularly useful in eliminating microbial organisms that grow within phagocytes e.g. *M. tuberculosis*, *Histoplasma capsulatum*.

ii) Oxygen-independent bactericidal mechanism. Some agents released from the granules of phagocytic cells do not require oxygen for bactericidal activity. These include lysosomal hydrolases, permeability increasing factors, defensins and cationic proteins.

iii) Nitric oxide mechanism. In addition to oxygen-dependent and oxygen-independent mechanisms, recently role of nitric oxide (NO) in inflammatory reaction has been emphasised. NO is produced by endothelial cells as well as by activated macrophages. In experimental animals, NO has been shown to have fungicidal and anti-parasitic action but its role in bactericidal activity in human beings is yet not clear.

CHEMICAL MEDIATORS OF INFLAMMATION

Also called as permeability factors or endogenous mediators of increased vascular permeability, these are a large and increasing number of endogenous compounds which can enhance vascular permeability. However, currently many chemical mediators have been identified which partake in other processes of acute inflammation as well e.g. vasodilatation, chemotaxis, fever, pain and cause tissue damage.

The substances acting as chemical mediators of inflammation may be released *from the cells, the plasma, or damaged tissue* itself. They are broadly classified into 2 groups:

- i) mediators released by cells; and
- ii) mediators originating from plasma.

Table 10.2 presents a list of chemical mediators of acute inflammation.

Chemical mediators involved in causing increased vascular permeability and consequent oedema of tissues are shown in Fig. 10.7.

TABLE 10.2: Chemical Mediators of Acute Inflammation.

I. CELL-DERIVED MEDIATORS

1. Vasoactive amines (Histamine, 5-hydroxytryptamine)
2. Arachidonic acid metabolites (Eicosanoids)
 - i. Metabolites via cyclo-oxygenase pathway (prostaglandins, thromboxane A_2 , prostacyclin)
 - ii. Metabolites via lipo-oxygenase pathway (5-HETE, leukotrienes)
3. Lysosomal components
4. Platelet activating factor
5. Cytokines (IL-1, TNF- α , TNF- β , IF- γ , chemokines)
6. Nitric oxide and oxygen metabolites

II. PLASMA-DERIVED MEDIATORS (PLASMA PROTEASES)

These are products of:

1. The kinin system
2. The clotting system
3. The fibrinolytic system
4. The complement system

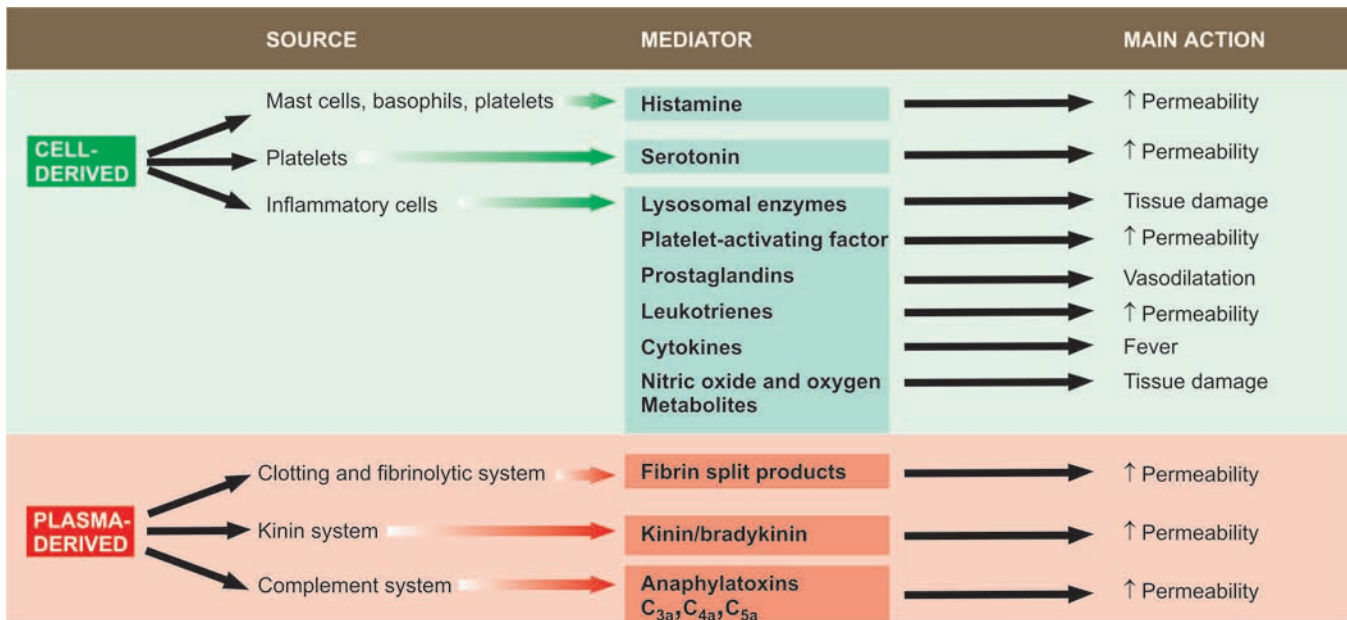


FIGURE 10.7

Chemical mediators of inflammation.

I. Cell-derived Mediators

1. **VASOACTIVE AMINES.** Two important pharmacologically active amines that have role in the early inflammatory response (first one hour) are histamine and 5-hydroxytryptamine (5-HT) or serotonin.

i) **Histamine.** It is stored in the granules of mast cells, basophils and platelets. Histamine is released from these cells by various agents as under:

- Stimuli or substances inducing acute inflammation e.g. heat, cold, irradiation, trauma, irritant chemicals, immunologic reactions etc.
- Anaphylatoxins like fragments of complement C_{3a} and C_{5a} which increase vascular permeability and cause oedema in tissues.
- Histamine-releasing factors from neutrophils, monocytes and platelets.
- Neuropeptides such as substance P.
- Interleukins.

The main actions of histamine are: vasodilatation, increased vascular (venular) permeability, itching and pain. Stimulation of mast cells and basophils also releases products of arachidonic acid metabolism including the release of *slow-reacting substances of anaphylaxis* (SRS-As). The SRS-As consist of various leukotrienes (LTC₄, LTD₄ and LTE₄).

ii) **5-Hydroxytryptamine (5-HT or serotonin).** It is present in tissues like chromaffin cells of GIT, spleen,

nervous tissue, mast cells and platelets. The actions of 5-HT are similar to histamine but it is a less potent mediator of increased vascular permeability and vasodilatation than histamine. It may be mentioned here that carcinoid tumour is a serotonin-secreting tumour.

2. **ARACHIDONIC ACID METABOLITES (EICOSANOIDS).** Arachidonic acid is a fatty acid, eicosatetraenoic acid, and its 2 main sources are:

- from diet directly; and
- conversion of essential fatty acid, linoleic acid to arachidonic acid.

Arachidonic acid must be first activated by stimuli or other mediators like C_{5a} so as to form arachidonic acid metabolites by one of the following 2 pathways: via cyclo-oxygenase pathway and via lipo-oxygenase pathway.

i) **Metabolites via cyclo-oxygenase pathway (prostaglandins, thromboxane A₂, prostacyclin).** The name 'prostaglandin' was first given to a substance found in human seminal fluid but now the same substance has been isolated from a number of other body tissues. Prostaglandins and related compounds are also called *autocoids*.

Cyclo-oxygenase is a fatty acid enzyme which acts on activated arachidonic acid to form prostaglandin endoperoxide (PGG₂). PGG₂ is enzymatically transformed into PGH₂ with generation of free radical of

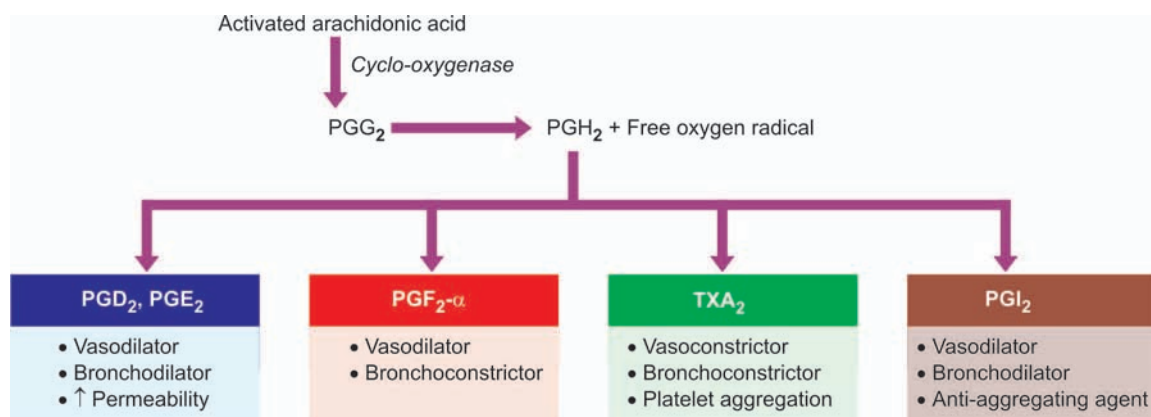


FIGURE 10.8

Arachidonic acid metabolites via cyclo-oxygenase pathway.

oxygen. PGH₂ is further acted upon by enzymes and results in formation of the following 3 metabolites (Fig. 10.8):

- Prostaglandins (PGD₂, PGE₂ and PGF₂-α)*. PGD₂ and PGE₂ act on blood vessels to cause increased venular permeability, vasodilatation and bronchodilatation and inhibit inflammatory cell function. PGF₂-α induces vasodilatation and bronchoconstriction.
- Thromboxane A₂ (TXA₂)*. It is a vasoconstrictor and broncho-constrictor and enhances inflammatory cell function by causing platelet aggregation.
- Prostacyclin (PGI₂)*. PGI₂ induces vasodilatation, bronchodilatation and inhibits inflammatory cell function by acting as anti-aggregating agent for platelets.

ii) Metabolites via lipo-oxygenase pathway (5-HETE, leukotrienes). The enzyme, lipo-oxygenase, acts on activated arachidonic acid to form hydroperoxy compound, 5-HPETE (hydroperoxy eico-satetraenoic acid) which on further peroxidation forms the following 2 metabolites (Fig. 10.9):

- 5-HETE* (hydroxy compound) which is a potent chemotactic agent for neutrophils.
- Leukotrienes (LT)* or slow-reacting substances of anaphylaxis (SRS-As) are so named as they were first isolated from leucocytes. Firstly, unstable leukotriene A₄ (LTA₄) is formed which is acted upon by enzymes to form LTB₄ (chemotactic for phagocytic cells and stimulates phagocytic cell adherence) while LTC₄, LTD₄ and LTE₄ have common actions by causing smooth muscle contraction and thereby

induce vasoconstriction, bronchoconstriction and increased vascular permeability.

3. LYSOSOMAL COMPONENTS. The inflammatory cells—neutrophils and monocytes, contain lysosomal granules which on release elaborate a variety of mediators of inflammation. These are as under:

i) Granules of neutrophils. These are of two types: specific or secondary, and azurophil or primary. The specific granules contain lactoferrin, lysozyme, alkaline phosphatase and collagenase while the large azurophil granules have myeloperoxidase, acid hydrolases and neutral proteases such as elastase, collagenase and proteinase.

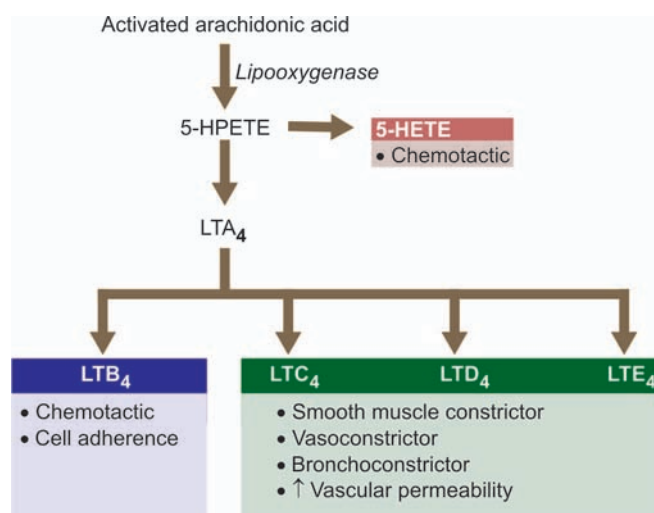


FIGURE 10.9

Arachidonic acid metabolites via lipoxygenase pathway.

Acid proteases act within the cell to cause destruction of bacteria in phagolysosome while neutral proteases attack on the extracellular constituents such as basement membrane, collagen, elastin, cartilage etc.

However, degradation of extracellular components like collagen, basement membrane, fibrin and cartilage by proteases results in harmful tissue destruction which is kept in check by antiproteases like α_1 -antitrypsin and α_2 -macroglobulin.

ii) Granules of monocytes and tissue macrophages. These cells on degranulation also release mediators of inflammation like acid proteases, collagenase, elastase and plasminogen activator. However, they are more active in chronic inflammation than acting as mediators of acute inflammation.

4. PLATELET ACTIVATING FACTOR (PAF). It is released from IgE-sensitised basophils or mast cells, other leucocytes, endothelium and platelets. Apart from its action on platelet aggregation and release reaction, the actions of PAF as mediator of inflammation are:

- increased vascular permeability;
- vasodilatation in low concentration and vasoconstriction otherwise;
- bronchoconstriction;
- adhesion of leucocytes to endothelium; and
- chemotaxis.

5. CYTOKINES. Cytokines are polypeptide substances produced by activated lymphocytes (*lymphokines*) and activated monocytes (*monokines*). These agents may act on 'self' cells producing them or on other cells. Currently, main cytokines acting as mediators of inflammation are: interleukin-1 (IL-1), tumour necrosis factor (TNF)- α and β , interferon (IF)- γ , and chemokines (IL-8, PF-4).

IL-1 and TNF- α are formed by activated macrophages while TNF- β and IF- γ are produced by activated T cells. The chemokines include interleukin 8 (released from activated macrophages) and platelet factor-4 from activated platelets, both of which are potent chemoattractant for inflammatory cells and hence their name.

The actions of various cytokines as mediator of inflammation are as under:

i) IL-1 and TNF- α , TNF- β induce endothelial effects in the form of increased leucocyte adherence, thrombogenicity, elaboration of other cytokines, fibroblastic proliferation and acute phase reactions.

ii) IF- γ causes activation of macrophages and neutrophils and is associated with synthesis of nitric acid synthase.

iii) Chemokines are a family of chemoattractants for inflammatory cells and include:

- IL-8 chemotactic for neutrophils;
- platelet factor-4 chemotactic for neutrophils, monocytes and eosinophils;
- MCP-1 chemotactic for monocytes; and
- eotaxin chemotactic for eosinophils.

6. NITRIC OXIDE AND OXYGEN METABOLITES.

Nitric oxide (NO) was originally described as vascular relaxation factor produced by endothelial cells. It has recently been included as a mediator of inflammatory responses since activated macrophages also produce NO during the oxidation of arginine by the action of enzyme, NO synthase.

NO plays the following role in inflammation:

- Vasodilatation
- Anti-platelet activating agent
- Possibly microbicidal action.

Oxygen-derived metabolites are released from activated neutrophils and macrophages and include superoxide oxygen (O_2^-), H_2O_2 , $OH^\cdot$ and toxic NO products. These oxygen-derived free radicals have the following action in inflammation:

- Endothelial cell damage and thereby increased vascular permeability.
- Activation of protease and inactivation of antiprotease causing tissue matrix damage.
- Damage to other cells.

The actions of free radicals are counteracted by antioxidants present in tissues and serum which play a protective role (page 30).

II. Plasma-derived Mediators (Plasma Proteases)

These include the various products derived from activation and interaction of 4 interlinked systems: kinin, clotting, fibrinolytic and complement. Each of these systems has its inhibitors and accelerators in plasma with negative and positive feedback mechanisms respectively.

Hageman factor (factor XII) of clotting system plays a key role in interactions of the four systems. Activation of factor XII *in vivo* by contact with basement membrane and bacterial endotoxins, and *in vitro* with glass or kaolin, leads to activation of clotting, fibrinolytic and kinin systems. In inflammation, activation of factor XII is brought about by contact of the factor leaking through the endothelial gaps. The end-products of the activated clotting, fibrinolytic and kinin systems activate the complement system that generate permeability factors. These permeability factors, in turn, further activate clotting system.

The inter-relationship between 4 systems is summarised in Fig. 10.10.

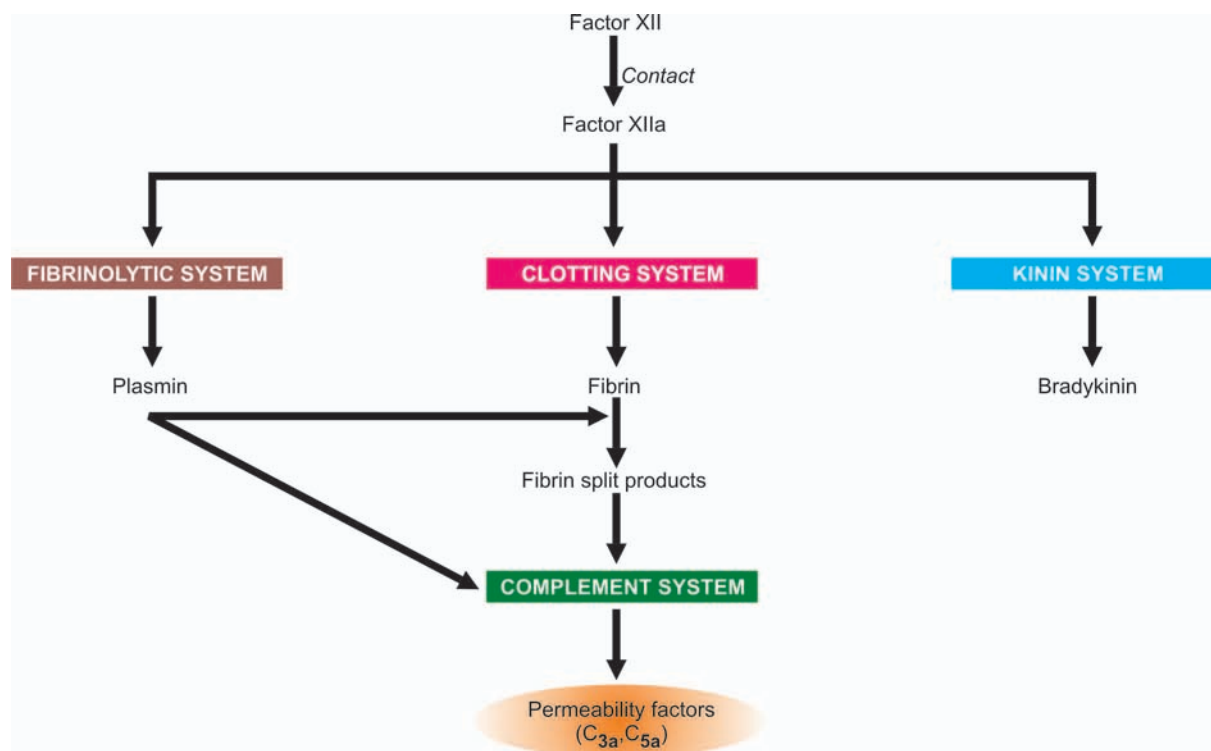


FIGURE 10.10

Inter-relationship between clotting, fibrinolytic, kinin and complement systems.

1. THE KININ SYSTEM. This system on activation by factor XIIa generates bradykinin, so named because of the slow contraction of smooth muscle it induces. First, kallikrein is formed from plasma prekallikrein by the action of prekallikrein activator which is a fragment of factor XIIa. Kallikrein then acts on high molecular weight kininogen to form bradykinin (Fig. 10.11).

Bradykinin acts in the early stage of inflammation and its effects include:

- smooth muscle contraction;
- vasodilatation;
- increased vascular permeability; and
- pain.

2. THE CLOTTING SYSTEM. Factor XIIa initiates the cascade of the clotting system resulting in formation of fibrinogen which is acted upon by thrombin to form fibrin and fibrinopeptides (Fig. 10.12).

The actions of fibrinopeptides in inflammation are:

- increased vascular permeability;
- chemotaxis for leucocyte; and
- anticoagulant activity.

3. THE FIBRINOLYTIC SYSTEM. This system is activated by plasminogen activator, the sources of which

include kallikrein of the kinin system, endothelial cells and leucocytes. Plasminogen activator acts on plasminogen present as component of plasma proteins to form plasmin. Further breakdown of fibrin by plasmin forms fibrinopeptides or fibrin split products (Fig. 10.13).

The actions of plasmin in inflammation are:

- activation of factor XII to form prekallikrein activator that stimulates the kinin system to generate bradykinin;

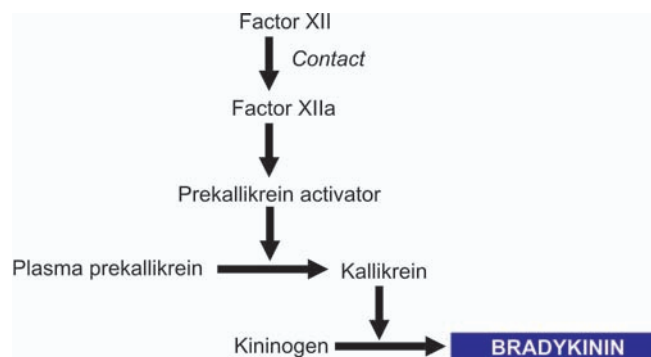


FIGURE 10.11

Pathway of the kinin system.

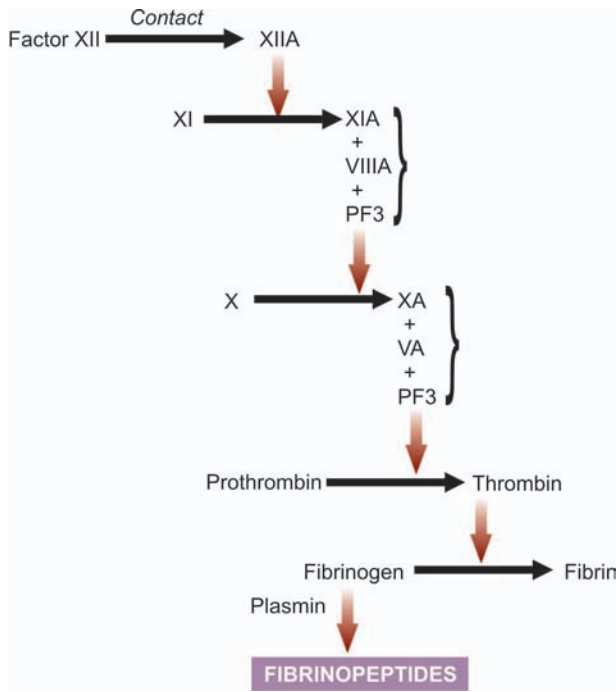


FIGURE 10.12

Pathway of the clotting system.

- splits off complement C_3 to form C_{3a} which is a permeability factor; and
- degrades fibrin to form fibrin split products which increase vascular permeability and are chemotactic to leucocytes.

4. THE COMPLEMENT SYSTEM. The activation of complement system can occur either:

- i) by *classic pathway* through antigen-antibody complexes; or
- ii) by *alternate pathway* via non-immunologic agents such as bacterial toxins, cobra venoms and IgA.

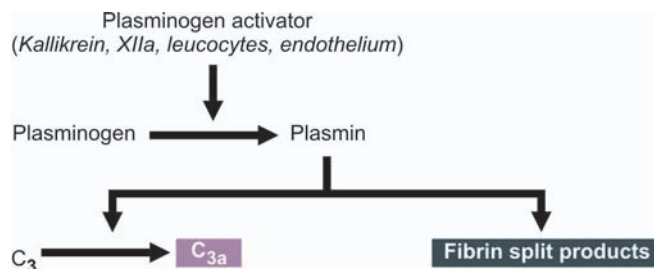


FIGURE 10.13

The activation of fibrinolytic system.

Complement system on activation by either of these two pathways yields anaphylatoxins C_{3a} , C_{4a} and C_{5a} , and membrane attack complex (MAC). The relative potencies of anaphylatoxins are in the descending sequence of C_{3a} , C_{5a} and C_{4a} .

The actions of *anaphylatoxins* in inflammation are:

- release of histamine from mast cells and basophils;
- increased vascular permeability causing oedema in tissues;
- C_{3b} augments phagocytosis; and
- C_{5a} is chemotactic for leucocytes.

The action of MAC is to cause pores in the cell membrane of the invading microorganisms.

REGULATION OF INFLAMMATION

The onset of inflammatory responses outlined above may have potentially damaging influence on the host tissues as evident in hypersensitivity conditions. Such self-damaging effects are kept in check by the host mechanisms so as to resolve inflammation. These include the following mechanisms:

i) Acute phase reactants. A variety of acute phase reactant (APR) proteins are released in plasma in response to tissue trauma and infection. These include the following:

- i) *Certain cellular protection factors* (α_1 -antitrypsin, α_1 -chymotrypsin, α_2 -antiplasmin, plasminogen activator inhibitor);
- ii) *Some coagulation proteins* (fibrinogen, plasminogen, von Willebrand factor, factor VIII);
- iii) *Transport proteins* (ceruloplasmin, haptoglobin);
- iv) *Immune agents* (serum amyloid A and P component, C-reactive protein); and
- v) *Stress proteins* (heat shock proteins—HSP, ubiquitin).

The APR are synthesised mainly in the liver and to some extent in macrophages. APR combined with systemic features of fever and leucocytosis is termed '*acute phase response*'. Deficient synthesis of APR leads to severe form of disease in chronic and repeated inflammatory responses.

ii) Corticosteroids. The endogenous glucocorticoids act as anti-inflammatory agents. Their levels are raised in infection and trauma by self-regulating mechanism.

iii) Free cytokine receptors. The presence of free receptors for cytokines in the serum correlates directly with disease activity.

iv) Suppressor T cells. A prohibition of suppressor T cells is seen which inhibits the function of T and B cells.

v) Anti-inflammatory chemical mediators. As already described, PGE_2 and prostacyclin have both pro-inflammatory as well as anti-inflammatory actions.

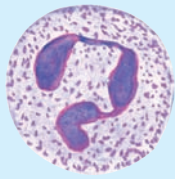
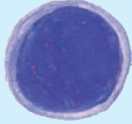
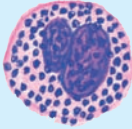
THE INFLAMMATORY CELLS

The cells participating in acute and chronic inflammation are circulating leucocytes, plasma cells and tissue macrophages. The structure, function and production of these cells are dealt with in detail in Chapter 34. Here, it is pertinent to describe the role of these cells in inflammation. Summary of their morphology, characteristics and functions is given in Table 10.3.

1. Polymorphonuclear Neutrophils (PMN)

Commonly called as neutrophils or polymorphs, these cells along with basophils and eosinophils are known as granulocytes due to the presence of granules in the cytoplasm. These granules contain many substances like proteases, myeloperoxidase, lysozyme, esterase, aryl sulfatase, acid and alkaline phosphatase, and cationic proteins. The diameter of neutrophils ranges from 10

TABLE 10.3: Morphology and Functions of Inflammatory Cells.

MORPHOLOGY	FEATURES	MEDIATORS
 A, POLYMORPH	i. Initial phagocytosis of bacteria and foreign body ii. Acute inflammatory cell	i. Primary granules (MPO, lysozyme, cationic proteins, acid hydrolases, elastase) ii. Secondary granules (lysozyme, alk. phosph, collagenase, lactoferrin) iii. Tertiary granules (gelatinase, cathepsin) iv. Reactive oxygen metabolites
 B, MONOCYTE/MACROPHAGE	i. Bacterial phagocytosis ii. Chronic inflammatory cell iii. Regulates lymphocyte response	i. Acid and neutral hydrolases (lysosomal) ii. Cationic protein iii. Phospholipase iv. Prostaglandins, leukotrienes v. IL-1
 C, LYMPHOCYTE	i. Humoral and cell-mediated immune responses ii. Chronic inflammatory cell iii. Regulates macrophage response	i. B cells: antibody production ii. T cells: delayed hypersensitivity, cytotoxicity
 D, PLASMA CELL	i. Derived from B cells ii. Chronic inflammatory cell	i. Antibody synthesis ii. Antibody secretion
 E, EOSINOPHIL	i. Allergic states ii. Parasitic infestations iii. Chronic inflammatory cell	i. Reactive oxygen metabolites ii. Lysosomal (major basic protein, cationic protein, eosinophil peroxidase, neurotoxin) iii. PGE ₂ synthesis
 F, BASOPHIL/MAST CELL	i. Receptor for IgE molecules ii. Electron-dense granules	i. Histamine ii. Leukotrienes iii. Platelet activating factor

to 15 μm and are actively motile (Table 10.3,A). These cells comprise 40-75% of circulating leucocytes and their number is increased in blood (neutrophilia) and tissues in acute bacterial infections. These cells arise in the bone marrow from stem cells (Chapter 34).

The functions of neutrophils in inflammation are as follows:

- i) **Initial phagocytosis** of micro-organisms as they form the first line of body defense in bacterial infection. The steps involved are adhesion of neutrophils to vascular endothelium, emigration through the vessel wall, chemotaxis, engulfment, degranulation, killing and degradation of the foreign material.
- ii) **Engulfment** of antigen-antibody complexes and non-microbial material.
- iii) **Harmful effect** of neutrophils is destruction of the basement membranes of glomeruli and small blood vessels.

2. Eosinophils

These are larger than neutrophils but are fewer in number, comprising 1 to 6% of total blood leucocytes (Table 10.3,E). Eosinophils share many structural and functional similarities with neutrophils like their production in the bone marrow, locomotion, phagocytosis, lobed nucleus and presence of granules in the cytoplasm containing a variety of enzymes, of which major basic protein and eosinophil cationic protein are the most important which have bactericidal and toxic action against helminthic parasites. However, granules of eosinophils are richer in myeloperoxidase than neutrophils and lack lysozyme. High level of steroid hormones leads to fall in number of eosinophils and even disappearance from blood.

The absolute number of eosinophils is increased in the following conditions and, thus, they partake in inflammatory responses associated with these conditions:

- i) allergic conditions;
- ii) parasitic infestations;
- iii) skin diseases; and
- iv) certain malignant lymphomas.

3. Basophils (Mast Cells)

The basophils comprise about 1% of circulating leucocytes and are morphologically and pharmacologically similar to mast cells of tissue. These cells contain coarse basophilic granules in the cytoplasm and a polymorphonuclear nucleus (Table 10.3,F). These granules are laden with heparin and histamine. Basophils and mast cells have receptors for IgE and degranulate when crosslinked with antigen.

The role of these cells in inflammation are:

- i) in immediate and delayed type of hypersensitivity reactions; and
- ii) release of histamine by IgE-sensitised basophils.

4. Lymphocytes

Next to neutrophils, these cells are most numerous of the circulating leucocytes (20-45%). Apart from blood, lymphocytes are present in large numbers in spleen, thymus, lymph nodes and mucosa-associated lymphoid tissue (MALT). They have scanty cytoplasm and consist almost entirely of nucleus (Table 10.3,C).

Besides their role in antibody formation (B lymphocytes) and in cell-mediated immunity (T lymphocytes), these cells participate in the following types of inflammatory responses:

- i) *In tissues*, they are dominant cells in chronic inflammation and late stage of acute inflammation.
- ii) *In blood*, their number is increased (lymphocytosis) in chronic infections like tuberculosis.

5. Plasma Cells

These cells are larger than lymphocytes with more abundant cytoplasm and an eccentric nucleus which has cart-wheel pattern of chromatin (Table 10.3,D). Plasma cells are normally not seen in peripheral blood. They develop from lymphocytes and are rich in RNA and γ -globulin in their cytoplasm. There is an inter-relationship between plasmacytosis and hyperglobulinaemia. These cells are most active in antibody synthesis.

Their number is increased in the following conditions:

- i) prolonged infection with immunological responses e.g. in syphilis, rheumatoid arthritis, tuberculosis;
- ii) hypersensitivity states; and
- iii) multiple myeloma.

6. Mononuclear-Phagocyte System (Reticuloendothelial System)

This cell system includes cells derived from 2 sources with common morphology, function and origin (Table 10.3,B). These are as under:

Blood monocytes. These comprise 4-8% of circulating leucocytes.

Tissue macrophages. These include the following cells in different tissues:

- i) Macrophages in inflammation.
- ii) Histiocytes, macrophages present in connective tissues.
- iii) Kupffer cells, macrophages of liver cells.

- iv) Alveolar macrophages (type II pneumocytes) in lungs.
- v) Macrophages/histiocytes of the bone marrow.
- vi) Tingible body cells of germinal centres of lymph nodes.
- vii) Littoral cells of splenic sinusoids.
- viii) Osteoclasts in the bones.
- ix) Microglial cells of the brain.
- x) Langerhans' cells/dendritic histiocytes of the skin.
- xi) Hoffbauer cells of the placenta.
- xii) Mesangial cells of glomerulus.

The mononuclear phagocytes are the scavenger cells of the body as well as participate in immune system of the body. Their functions in inflammation are given below while their role in the immune system are described on page 146.

Role of macrophages in inflammation. The functions of mononuclear-phagocyte cells are as under:

- i) *Phagocytosis* (cell eating) and *pinocytosis* (cell drinking).
- ii) *Macrophages on activation* by lymphokines released by T lymphocytes or by non-immunologic stimuli elaborate a variety of biologically active substances as under:
 - a) Proteases like collagenase and elastase which degrade collagen and elastic tissue.
 - b) Plasminogen activator which activates the fibrinolytic system.
 - c) Products of complement.
 - d) Some coagulation factors (factor V and thromboplastin) which convert fibrinogen to fibrin.
 - e) Chemotactic agents for other leucocytes.
 - f) Metabolites of arachidonic acid.
 - g) Growth promoting factors for fibroblasts, blood vessels and granulocytes.
 - h) Cytokines like interleukin-1 and tumour necrosis factor.
 - i) Oxygen-derived free radicals.

7. Giant Cells

A few examples of multinucleate giant cells exist in normal tissues (e.g. osteoclasts in the bones, trophoblasts in placenta, megakaryocytes in the bone marrow). However, in chronic inflammation when the macrophages fail to deal with particles to be removed, they fuse together and form multinucleated giant cells. Besides, morphologically distinct giant cells appear in some tumours also. Some of the common types of giant cells are described below:

A. Giant cells in inflammation:

i) *Foreign body giant cells.* These contain numerous nuclei (up to 100) which are uniform in size and shape and resemble the nuclei of macrophages. These nuclei are scattered throughout the cytoplasm (Fig. 10.14,A). These are seen in chronic infective granulomas, leprosy and tuberculosis.

ii) *Langhans' giant cells.* These are seen in tuberculosis and sarcoidosis. Their nuclei are like the nuclei of macrophages and epithelioid cells. These nuclei are arranged either around the periphery in the form of horseshoe or ring or are clustered at the two poles of the giant cell (Fig. 10.14,B).

iii) *Touton giant cells.* These multinucleated cells have vacuolated cytoplasm due to lipid content e.g. in xanthoma (Fig. 10.14,C).

iv) *Aschoff giant cells.* These multinucleate giant cells are derived from cardiac histiocytes and are seen in rheumatic nodule (Chapter 25).

B. Giant cells in tumours:

i) *Anaplastic cancer giant cells.* These are larger, have numerous nuclei which are hyperchromatic and vary in size and shape (Fig. 10.14,D). These giant cells are not derived from macrophages but are formed from dividing nuclei of the neoplastic cells e.g. carcinoma of liver, various soft tissue sarcomas etc.

ii) *Reed-Sternberg cells.* These are also malignant tumour giant cells which are generally binucleate and are seen in various histologic types of Hodgkin's lymphomas (Fig. 10.14,E).

iii) *Giant cell tumour of bone.* This tumour of the bones has uniform distribution of osteoclastic giant cells spread in the stroma (Fig. 10.14,F).

FACTORS DETERMINING VARIATION IN INFLAMMATORY RESPONSE

Although acute inflammation is typically characterised by vascular and cellular events with emigration of neutrophilic leucocytes, not all examples of acute inflammation show infiltration by neutrophils. On the other hand, some chronic inflammatory conditions are characterised by neutrophilic infiltration. For example, *typhoid fever* is an example of acute inflammatory process but the cellular response in it is lymphocytic; *osteomyelitis* is an example of chronic inflammation but the cellular response in this condition is mainly neutrophilic.

The morphologic variation in inflammation depends upon a number of factors and processes. These are discussed below:

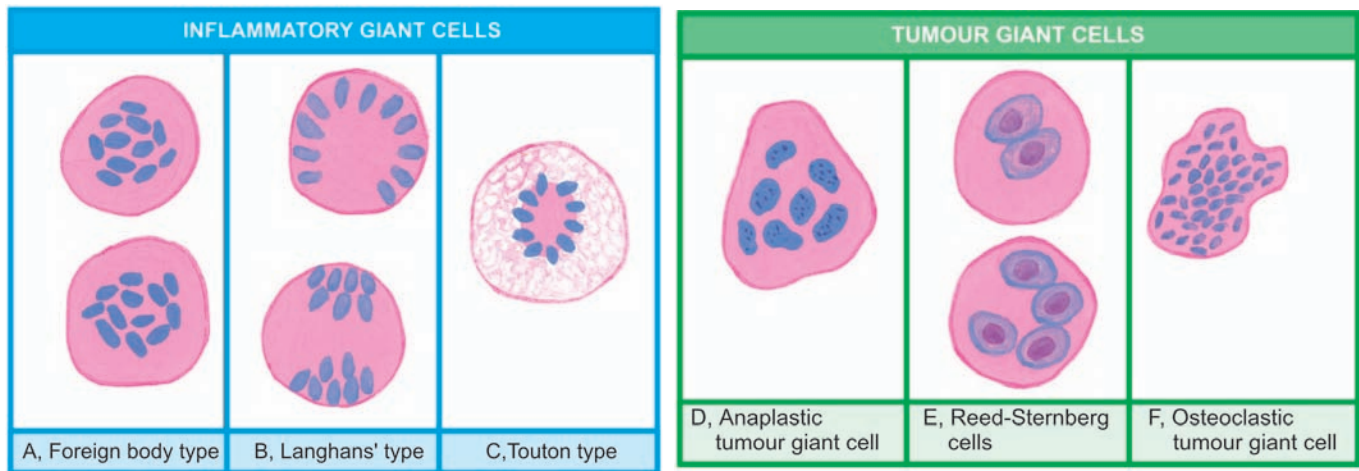


FIGURE 10.14

Giant cells of various types. A, Foreign body giant cell with uniform nuclei dispersed throughout the cytoplasm. B, Langhans' giant cells with uniform nuclei arranged peripherally or clustered at the two poles. C, Touton giant cell with circular pattern of nuclei and vacuolated cytoplasm. D, Anaplastic tumour giant cell with nuclei of variable size and shape. E, Reed-Sternberg cells. F, Osteoclastic tumour giant cell.

1. Factors Involving the Organisms

i) Type of injury and infection. For example, skin reacts to herpes simplex infection by formation of vesicle and to streptococcal infection by formation of boil; lung reacts to pneumococci by occurrence of lobar pneumonia while to tubercle bacilli it reacts by granulomatous inflammation.

ii) Virulence. Many species and strains of organisms may have varying virulence e.g. the three strains of *C. diphtheriae* (*gravis*, *intermedius* and *mitis*) produce the same diphtherial exotoxin but in different amount.

iii) Dose. The concentration of organism in small doses produces usually local lesions while larger dose results in more severe spreading infections.

iv) Portal of entry. Some organisms are infective only if administered by particular route e.g. *Vibrio cholerae* is not pathogenic if injected subcutaneously but causes cholera if swallowed.

v) Product of organisms. Some organisms produce enzymes that help in spread of infections e.g. hyaluronidase by *Clostridium welchii*, streptokinase by streptococci, staphylokinase and coagulase by staphylococci.

2. Factors Involving the Host

i) General health of host. For example, starvation, haemorrhagic shock, chronic debilitating diseases like diabetes mellitus, alcoholism etc render the host more susceptible to infections.

ii) Immune state of host. Immunodeficiency helps in spread of infections rapidly e.g. in AIDS.

iii) Leukopenia. Patients with low WBC count with neutropenia or agranulocytosis develop spreading infection.

iv) Site or type of tissue involved. For example, the lung has loose texture as compared to bone and, thus, both tissues react differently to acute inflammation.

v) Local host factors. For instance, ischaemia, presence of foreign bodies and chemicals cause necrosis and are thus harmful.

3. Type of Exudation

The appearance of escaped plasma determines the morphologic type of inflammation as under:

i) Serous, when the fluid exudate resembles serum or is watery e.g. pleural effusion in tuberculosis, blister formation in burns.

ii) Fibrinous, when the fibrin content of the fluid exudate is high e.g. in pneumococcal and rheumatic pericarditis.

iii) Purulent or suppurative exudate is formation of creamy pus as seen in infection with pyogenic bacteria e.g. abscess, acute appendicitis.

iv) Haemorrhagic, when there is vascular damage e.g. acute haemorrhagic pneumonia in influenza.

v) Catarrhal, when the surface inflammation of epithelium produces increased secretion of mucous e.g. common cold.

4. Cellular Proliferation

Variable cellular proliferation is seen in different types of inflammations.

- i) There is no significant cellular proliferation in **acute bacterial infections** except in typhoid fever in which there is intestinal lymphoid hyperplasia.
- ii) **Viral infections** have the ability to stimulate cellular proliferation e.g. epidermal cell proliferation in herpes simplex, chickenpox and smallpox.
- iii) In **rapidly progressive glomerulonephritis**, there is proliferation of glomerular capsular epithelial cells resulting in formation of 'crescents'.
- iv) In **chronic inflammation**, cellular proliferation of macrophages, fibroblasts and endothelial cells occurs.

5. Necrosis

The extent and type of necrosis in inflammation is variable.

- i) In **gas gangrene**, there is extensive necrosis with discoloured and foul smelling tissues.
- ii) In **acute appendicitis**, there is necrosis as a result of vascular obstruction.
- iii) In **chronic inflammation** such as tuberculosis, there is characteristic caseous necrosis.

MORPHOLOGY OF ACUTE INFLAMMATION

Inflammation of an organ is usually named by adding the suffix-*itis* to its Latin name e.g. appendicitis, hepatitis, cholecystitis, meningitis etc. A few morphologic varieties of acute inflammation are described below (COLOUR PLATE IV: CL 13):

1. PSEUDOMEMBRANOUS INFLAMMATION. It is inflammatory response of mucous surface (oral, respiratory, bowel) to toxins of diphtheria or irritant gases. As a result of denudation of epithelium, plasma exudes on the surface where it coagulates, and together with necrosed epithelium, forms false membrane that gives this type of inflammation its name.

2. ULCER. Ulcers are local defects on the surface of an organ produced by inflammation. Common sites for ulcerations are the stomach, duodenum, intestinal ulcers in typhoid fever, intestinal tuberculosis, bacillary and amoebic dysentery, ulcers of legs due to varicose veins etc. In the acute stage, there is infiltration by polymorphs with vasodilatation while long-standing ulcers develop infiltration by lymphocytes, plasma cells and macrophages with associated fibroblastic proliferation and scarring.

3. SUPPURATION (ABSCESS FORMATION). When acute bacterial infection is accompanied by intense neutrophilic infiltrate in the inflamed tissue, it results in tissue necrosis. A cavity is formed which is called an abscess and contains purulent exudate or pus and the process of abscess formation is known as suppuration. The bacteria which cause suppuration are called pyogenic.

Microscopically, pus is creamy or opaque in appearance and is composed of numerous dead as well as living neutrophils, some red cells, fragments of tissue debris and fibrin. In old pus, macrophages and cholesterol crystals are also present (Fig. 10.15).

An abscess may be discharged to the surface due to increased pressure inside or may require drainage by the surgeon. Due to tissue destruction, resolution does not occur but instead healing by fibrous scarring takes place.

Some of the common examples of abscess formation are as under:

- i) *Boil or furruncle* which is an acute inflammation via hair follicles in the dermal tissues.
- ii) *Carbuncle* is seen in untreated diabetics and occurs as a loculated abscess in the dermis and soft tissues of the neck.

4. CELLULITIS. It is a diffuse inflammation of soft tissues resulting from spreading effects of substances like hyaluronidase released by some bacteria.

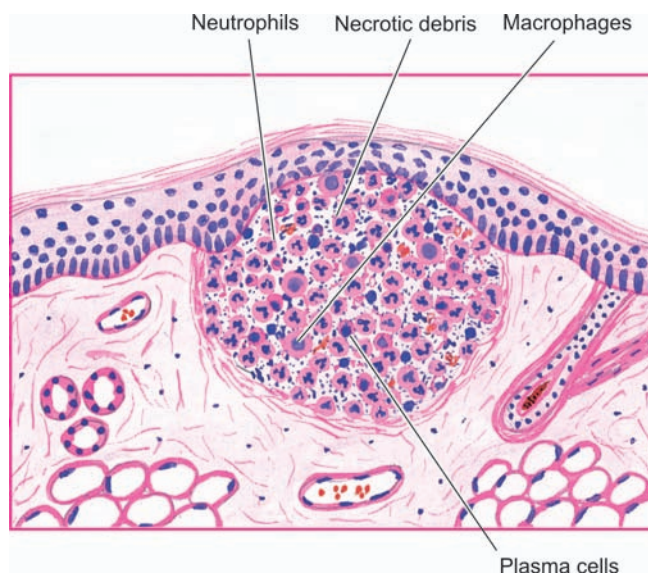


FIGURE 10.15

An abscess in the skin. It contains pus composed of necrotic tissue, debris, fibrin, RBCs and dead and living neutrophils. Some macrophages are seen at the periphery.

5. BACTERIAL INFECTION OF THE BLOOD. This includes the following 3 conditions:

i) **Bacteraemia** is defined as presence of small number of bacteria in the blood which do not multiply significantly. They are commonly not detected by direct microscopy. Blood culture is done for their detection e.g. infection with *Salmonella typhi*, *Escherichia coli*, *Streptococcus viridans*.

ii) **Septicaemia** means presence of rapidly multiplying, highly pathogenic bacteria in the blood e.g. pyogenic cocci, bacilli of plague etc. Septicaemia is generally accompanied by systemic effects like toxæmia, multiple small haemorrhages, neutrophilic leucocytosis and disseminated intravascular coagulation (DIC).

iii) **Pyæmia** is the dissemination of small septic thrombi in the blood which cause their effects at the site where they are lodged. This can result in pyæmic abscesses or septic infarcts.

- a) *Pyæmic abscesses* are multiple small abscesses in various organs such as in cerebral cortex, myocardium, lungs and renal cortex, resulting from very small emboli fragmented from septic thrombus. Microscopy of pyæmic abscess shows a central zone of necrosis containing numerous bacteria, surrounded by a zone of suppuration and an outer zone of acute inflammatory cells (Fig. 10.16,A).
- b) *Septic infarcts* result from lodgement of larger fragments of septic thrombi in the arteries with relatively larger foci of necrosis, suppuration and acute inflammation e.g. septic infarcts of the lungs, liver, brain, and kidneys from septic thrombi of leg veins or from acute bacterial endocarditis (Fig. 10.16,B).

SYSTEMIC EFFECTS OF ACUTE INFLAMMATION

The account of acute inflammation given up to now above is based on local tissue responses. However, acute inflammation is associated with systemic effects as well. These include fever, leucocytosis and lymphangitis-lymphadenitis.

1. Fever occurs due to bacteraemia. It is thought to be mediated through release of factors like prostaglandins, interleukin-1 and tumour necrosis factor in response to infection.

2. Leucocytosis commonly accompanies the acute inflammatory reactions, usually in the range of 15,000-20,000/ μ l. When the counts are higher than this with 'shift to left' of myeloid cells, the blood picture is described as leukaemoid reaction. Usually, in bacterial infections there is neutrophilia; in viral infections lymphocytosis; and in parasitic infestations, eosino-

philia. Typhoid fever, an example of acute inflammation, however, induces leucopenia with relative lymphocytosis.

3. Lymphangitis-lymphadenitis is one of the important manifestations of localised inflammatory injury. The lymphatics and lymph nodes that drain the inflamed tissue show reactive inflammatory changes in the form of lymphangitis and lymphadenitis. This response represents either a nonspecific reaction to mediators released from inflamed tissue or is an immunologic response to a foreign antigen. The affected lymph nodes may show hyperplasia of lymphoid follicles (follicular hyperplasia) and proliferation of mononuclear phagocytic cells in the sinuses of lymph node (sinus histiocytosis) (Chapter 37).

4. Shock may occur in severe cases. Massive release of cytokine $\text{TNF-}\alpha$, a mediator of inflammation, in response to severe tissue injury or infection results in profuse systemic vasodilatation, increased vascular permeability and intravascular volume loss. The net effect of these changes is hypotension and shock. Systemic activation of coagulation pathway may occur leading to microthrombi throughout the body and result in disseminated intravascular coagulation (DIC), bleeding and death.

FATE OF ACUTE INFLAMMATION

The acute inflammatory process can culminate in one of the following 4 outcomes:

1. Resolution
2. Healing by scarring
3. Progression to suppuration
4. Progression to chronic inflammation.

1. Resolution. It means complete return to normal tissue following acute inflammation. This occurs when tissue changes are slight and the cellular changes are reversible e.g. resolution in lobar pneumonia.

2. Healing by scarring. This takes place when the tissue destruction in acute inflammation is extensive so that there is no tissue regeneration but actually there is healing by fibrosis.

3. Suppuration. When the pyogenic bacteria causing acute inflammation result in severe tissue necrosis, the process progresses to suppuration. Initially, there is intense neutrophilic infiltration. Subsequently, mixture of neutrophils, bacteria, fragments of necrotic tissue, cell debris and fibrin comprise pus which is contained in a cavity to form an abscess. The abscess, if not drained, may get organised by dense fibrous tissue, and in time, get calcified.

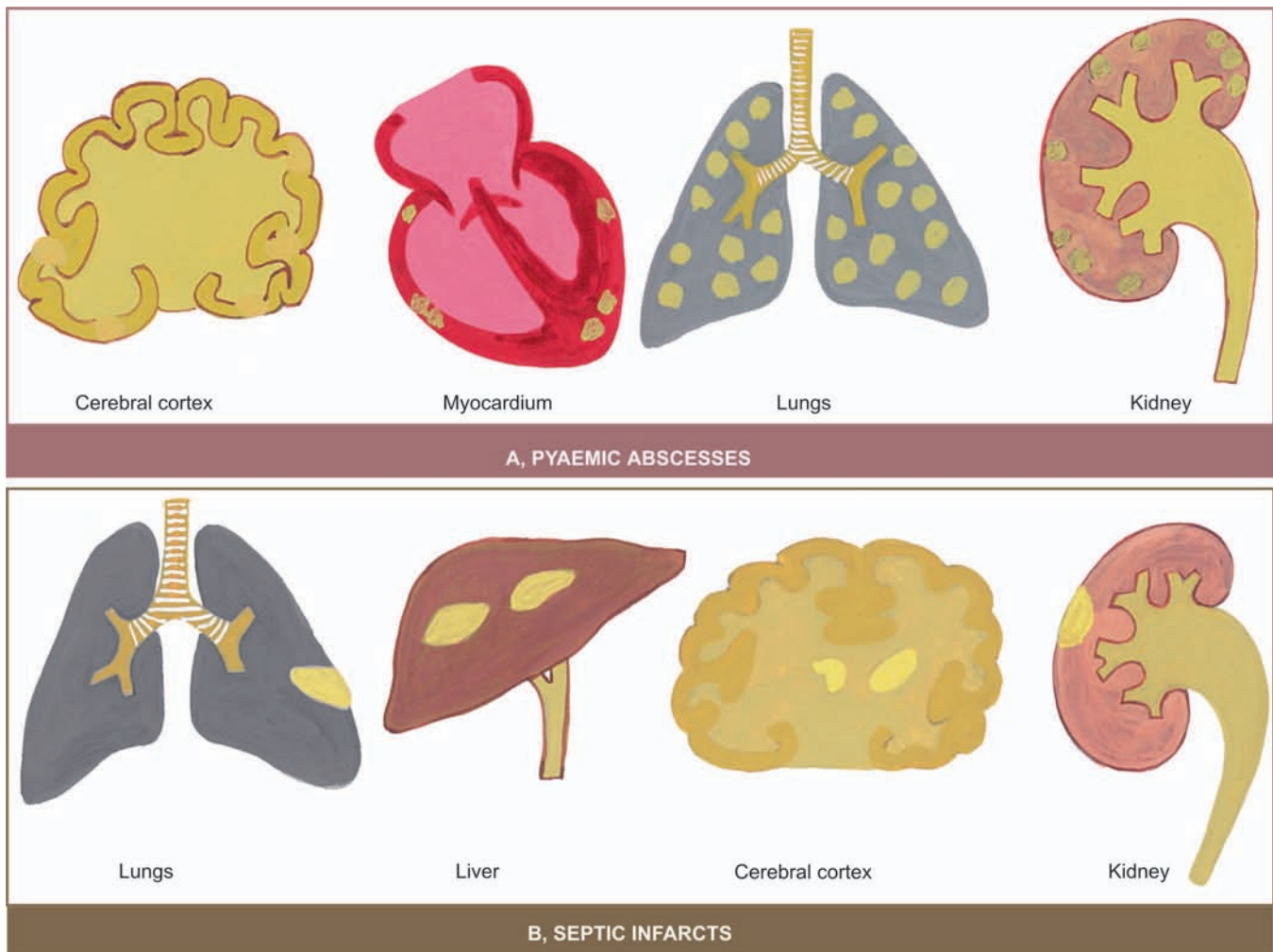


FIGURE 10.16

Sequelae of pyaemia.

4. Chronic inflammation. The acute inflammation may progress to chronic inflammation in which the processes of inflammation and healing proceed side by side.

CHRONIC INFLAMMATION

DEFINITION AND CAUSES. Chronic inflammation is defined as prolonged process in which tissue destruction and inflammation occur at the same time.

Chronic inflammation can be caused by one of the following 3 ways:

1. Chronic inflammation following acute inflammation. When the tissue destruction is extensive, or the bacteria survive and persist in small numbers at the site of acute inflammation e.g. in osteomyelitis, pneumonia terminating in lung abscess.

2. Recurrent attacks of acute inflammation. When repeated bouts of acute inflammation culminate in chronicity of the process e.g. in recurrent urinary tract infection leading to chronic pyelonephritis, repeated acute infection of gallbladder leading to chronic cholecystitis.

3. Chronic inflammation starting *de novo*. When the infection with organisms of low pathogenicity is chronic from the beginning e.g. infection with *Mycobacterium tuberculosis*.

GENERAL FEATURES OF CHRONIC INFLAMMATION

Though there may be differences in chronic inflammatory response depending upon the tissue involved and causative organisms, there are some basic simi-

larities amongst various types of chronic inflammation. Following general features characterise any chronic inflammation:

- 1. MONONUCLEAR CELL INFILTRATION.** Chronic inflammatory lesions are infiltrated by mononuclear inflammatory cells like phagocytes and lymphoid cells. Phagocytes are represented by circulating monocytes, tissue macrophages, epithelioid cells and sometimes, multinucleated giant cells. The macrophages comprise the most important cells in chronic inflammation. These may appear at the site of chronic inflammation from:
 - i) chemotactic factors and adhesion molecules for continued infiltration of macrophages;
 - ii) local proliferation of macrophages; and
 - iii) longer survival of macrophages at the site of inflammation.

The blood monocytes on reaching the extravascular space transform into tissue macrophages. Besides the role of macrophages in phagocytosis, they may get activated in response to stimuli such as cytokines (lymphokines) and bacterial endotoxins. On activation, macrophages release several biologically active substances e.g. acid and neutral proteases, oxygen-derived reactive metabolites and cytokines. These products bring about tissue destruction, neovascularisation and fibrosis.

Other chronic inflammatory cells include lymphocytes, plasma cells, eosinophils and mast cells. In chronic inflammation, lymphocytes and macrophages influence each other and release mediators of inflammation.

- 2. TISSUE DESTRUCTION OR NECROSIS.** Tissue destruction and necrosis are central feature of most forms of chronic inflammatory lesions. This is brought about by activated macrophages which release a variety of biologically active substances e.g. protease, elastase, collagenase, lipase, reactive oxygen radicals, cytokines (IL-1, IL-8, TNF), nitric oxide, angiogenesis growth factor etc.

- 3. PROLIFERATIVE CHANGES.** As a result of necrosis, proliferation of small blood vessels and fibroblasts is stimulated resulting in formation of inflammatory granulation tissue. Eventually, healing by fibrosis and collagen laying takes place.

SYSTEMIC EFFECTS OF CHRONIC INFLAMMATION

Chronic inflammation is associated with following systemic features:

- 1. Fever.** Invariably there is mild fever, often with loss of weight and weakness.
- 2. Anaemia.** As discussed in Chapter 33, chronic inflammation is accompanied by anaemia of varying degree.
- 3. Leucocytosis.** As in acute inflammation, chronic inflammation also has leucocytosis but generally there is relative lymphocytosis in these cases.
- 4. ESR.** ESR is elevated in all cases of chronic inflammation.
- 5. Amyloidosis.** Long-term cases of chronic suppurative inflammation may develop secondary systemic (AA) amyloidosis.

TYPES OF CHRONIC INFLAMMATION

Conventionally, chronic inflammation is subdivided into 2 types:

- 1. Non-specific,** when the irritant substance produces a non-specific chronic inflammatory reaction with formation of granulation tissue and healing by fibrosis e.g. chronic osteomyelitis, chronic ulcer.
- 2. Specific,** when the injurious agent causes a characteristic histologic tissue response e.g. tuberculosis, leprosy, syphilis.

However, for a more descriptive classification, histological features are used for classifying chronic inflammation into 2 corresponding types:

- 1. Chronic non-specific inflammation.** It is characterised by non-specific inflammatory cell infiltration e.g. chronic osteomyelitis, lung abscess. A variant of this type of chronic inflammatory response is chronic suppurative inflammation in which infiltration by polymorphs and abscess formation are additional features e.g. actinomycosis.
- 2. Chronic granulomatous inflammation.** It is characterised by formation of granulomas e.g. tuberculosis, leprosy, syphilis, actinomycosis, sarcoidosis etc.



11

Granulomatous Inflammation

Granuloma is defined as a circumscribed, tiny lesion, about 1 mm in diameter, composed predominantly of collection of modified macrophages called epithelioid cells, and rimmed at the periphery by lymphoid cells. The word '*granuloma*' is derived from *granule* meaning circumscribed granule-like lesion, and *-oma* which is a suffix commonly used for true tumours but here indicates inflammatory mass or collection of macrophages.

Epithelioid cells, so called because of their epithelial cell-like appearance, are modified macrophages/histiocytes which are somewhat elongated, having pale-staining abundant cytoplasm, vesicular and lightly-staining slipper-shaped nucleus, while the cell membrane of adjacent epithelioid cells is closely apposed due to hazy cell outline. Epithelioid cells are weakly phagocytic.

Besides the presence of epithelioid cells and lymphoid cells, granulomas may have giant cells, necrosis and fibrosis:

1. The giant cells are formed by fusion of adjacent epithelioid cells and may have 20 or more nuclei. These nuclei may be arranged at the periphery like horse-shoe or ring or clustered at the two poles (Langhans' giant cells), or they may be present centrally (foreign body giant cells). The former are commonly seen in tuberculosis while the latter are common in foreign body tissue reactions. Like epithelioid cells these giant cells are weakly phagocytic but produce secretory products which help in removing the invading agents.

2. Necrosis may be a feature of some granulomatous conditions e.g. central caseation necrosis of tuberculosis, so called because of cheese-like appearance and consistency of necrosis.

3. Fibrosis is due to proliferation of fibroblasts at the periphery of granuloma.

The following two factors favour the formation of granulomas:

- i) *Presence of poorly digestible irritant* which may be organisms like *Mycobacterium tuberculosis*, particles of talc, etc.
- ii) *Presence of cell-mediated immunity* to the irritant, implying thereby the role of hypersensitivity in granulomatous inflammation.

The mechanism of evolution of granuloma is graphically depicted in Fig. 11.1.

The classical example of granulomatous inflammation is the tissue response to tubercle bacilli which is called as *tubercle* seen in tuberculosis (described below). A fully-developed tubercle is about 1 mm in diameter with central area of caseation necrosis, surrounded by epithelioid cells and one to several multinucleated giant cells (commonly Langhans' type),

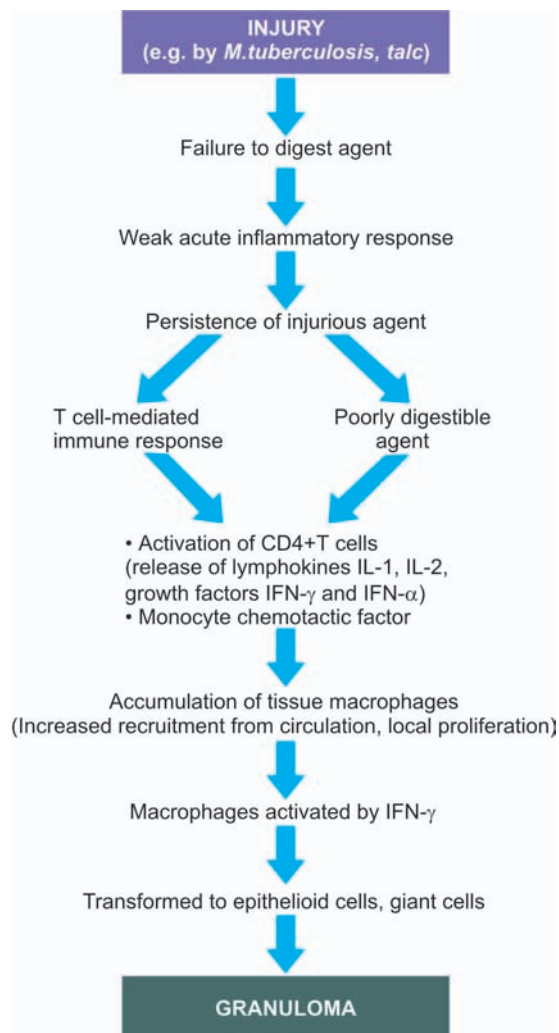


FIGURE 11.1

Mechanism of evolution of a granuloma (IL=interleukin; IFN=interferon).

surrounded at the periphery by lymphocytes and bounded by fibroblasts and fibrous tissue.

EXAMPLES OF GRANULOMATOUS INFLAMMATION

Granulomatous inflammation is typical of reaction to poorly digestible agents elicited by tuberculosis, leprosy, fungal infections, schistosomiasis, foreign particles etc. A comprehensive list of important examples of granulomatous conditions, their etiologic agents and salient features is given in Table 11.1. The principal examples (marked with asterisk in the table) are discussed below.

TUBERCULOSIS

Tissue response in tuberculosis represents classical example of chronic granulomatous inflammation in humans.

CAUSATIVE ORGANISM. Tubercle bacillus or Koch's bacillus (named after discovery of the organism by Robert Koch in 1882) called *Mycobacterium tuberculosis* causes tuberculosis in the lungs and other tissues of the human body. The organism is a strict aerobe and thrives best in tissues with high oxygen tension like in the apex of the lung.

The organism has 5 distinct pathogenic strains: *hominis*, *bovis*, *avium*, *murine*, and *cold-blooded vertebrate strain*. A sixth non-pathogenic strain, *M. smegmatis*, is found in the smegma and as contaminant in the urine of both men and women. Out of these, the first two strains (*hominis* and *bovis*) are definitely infective to human beings while infection with avium strain (*M. avium-intracellulare*) is common in patients with AIDS.

M. tuberculosis hominis is a slender rod-like bacillus, 4 µm in length, and can be demonstrated by the following methods:

1. *Acid fast (Ziehl-Neelsen) staining.* The acid fastness of the tubercle bacilli is due to waxy content in the cell wall of the organism making it impermeable to the usual stains. It takes up stain by heated carbol fuchsin and resists decolourisation by acids and alcohols (acid fast and alcohol fast) and can be decolourised by 20% sulphuric acid.
2. *Fluorescent dye methods.*
3. *Culture of the organism in Lowenstein-Jensen (L.J.) medium for 6 weeks.*
4. *Guinea pig inoculation method by subcutaneous injection of the organisms.*

ATYPICAL MYCOBACTERIA. Occasionally, human tuberculosis may be caused by atypical mycobacteria which are non-pathogenic to guinea pigs and resistant to usual anti-tubercular drugs.

There are 4 groups of atypical mycobacteria:

Group I: Photochromogens. These organisms produce yellow pigment in the culture grown in light.

Group II: Scotochromogens. Pigment is produced, whether the growth is in light or in dark.

Group III: Non-chromogens. No pigment is produced by the bacilli and the organism is closely related to avium bacillus.

Group IV: Rapid growers. These organisms grow fast in the culture but are less pathogenic than others.

The infection by atypical mycobacteria is acquired directly from the environment, unlike person-to-person transmission of classical tuberculosis and the disease produced is known as *atypical mycobacteriosis*. The lesions produced may be granulomas, nodular collection of foamy cells, or acute inflammation.

Five patterns of the disease are recognised:

- i) Pulmonary disease produced by *M. kansasii* or *M. avium-intracellulare*.
- ii) Lymphadenitis caused by *M. avium-intracellulare* or *M. scrofulaceum*.
- iii) Ulcerated skin lesion produced by *M. ulcerans* or *M. marinum*.
- iv) Abscess caused by *M. fortuitum* or *M. chelonae*.
- v) Bacteraemias by *M. avium-intracellulare* as seen in immunosuppressed patients of AIDS.

INCIDENCE. In spite of great advances in chemotherapy and immunology, tuberculosis still continues to be worldwide in distribution, more common in poorer countries of Africa, Latin America and Asia. Other factors contributing to higher incidence of tuberculosis are malnutrition, inadequate medical care, poverty, crowding, chronic debilitating conditions like uncontrolled diabetes, alcoholism and immunocompromised states like AIDS. However, the exact incidence of disease cannot be determined as all patients infected with *M. tuberculosis* may not develop the clinical disease and many remain reactive to tuberculin without developing symptomatic disease.

MODE OF TRANSMISSION. Human beings acquire infection with tubercle bacilli by one of the following routes:

1. *Inhalation* of organisms present in fresh cough droplets or in dried sputum from an open case of pulmonary tuberculosis.
2. *Ingestion* of the organisms leads to development of tonsillar or intestinal tuberculosis. This mode of infection of human tubercle bacilli is from self-swallowing of infected sputum of an open case of pulmonary tuberculosis, or ingestion of bovine tubercle bacilli from milk of diseased cows.

TABLE 11.1: Principal Granulomatous Conditions.

CONDITIONS	ETIOLOGIC AGENT	SPECIAL CHARACTERISTICS
I. BACTERIAL		
1. <i>Tuberculosis*</i>	<i>Mycobacterium tuberculosis</i>	Tuberculous granulomas with central caseation necrosis; acid-fast bacilli.
2. <i>Leprosy*</i>	<i>Mycobacterium leprae</i>	Foamy histiocytes with acid-fast bacilli (lepromatous); epithelioid cell granulomas (tuberculoid).
3. <i>Syphilis*</i>	<i>Treponema pallidum</i>	Gummas composed of histiocytes; plasma cell infiltration; central necrosis.
4. <i>Granuloma inguinale (Donovanosis)</i>	<i>C. donovani</i> (Donovan body)	Anal and genital lesions; macrophages and neutrophils show Donovan bodies.
5. <i>Brucellosis (Mediterranean fever)</i>	<i>Brucella abortus</i>	Dairy infection to humans; enlarged reticuloendothelial organs (lymph nodes, spleen, bone marrow); non-specific granulomas.
6. <i>Cat scratch disease</i>	<i>Coccobacillus</i>	Lymphadenitis; reticuloendothelial hyperplasia; granulomas with central necrosis and neutrophils.
7. <i>Tularaemia (Rabbit fever)</i>	<i>Francisella (Pasteurella) tularensis</i>	Necrosis and suppuration (acute); tubercles hard or with minute central necrosis (chronic).
8. <i>Glanders</i>	<i>Actinobacillus mallei</i>	Infection from horses and mules; subcutaneous lesions and lymphadenitis; infective granulomas.
II. FUNGAL		
1. <i>Actinomycosis* (bacterial)</i>	<i>Actinomycetes israelii</i>	Cervicofacial, abdominal and thoracic lesions; granulomas and abscesses with draining sinuses; sulphur granules.
2. <i>Blastomycosis</i>	<i>Blastomyces dermatitidis</i>	Cutaneous, systemic and lung lesions; suppuration; ulceration and granulomas.
3. <i>Cryptococcosis</i>	<i>Cryptococcus neoformans</i>	Meninges, lungs and systemic distribution; organism yeast-like with clear capsule.
4. <i>Coccidioidomycosis</i>	<i>Coccidioides immitis</i>	Meninges, lungs and systemic distribution; granulomas and abscesses; organism cyst containing endospores.
III. PARASITIC		
<i>Schistosomiasis (Bilharziasis)</i>	<i>Schistosoma mansoni, haematobium, japonicum</i>	Eggs and granulomas in gut, liver, lung; schistosome pigment; eosinophils in blood and tissue.
IV. MISCELLANEOUS		
1. <i>Sarcoidosis*</i>	Unknown	Non-caseating granulomas (hard tubercles); asteroid and Schaumann bodies in giant cells.
2. <i>Crohn's disease (Regional enteritis)</i>	Unknown ? Bacteria, ?? Viruses	Transmural chronic inflammatory infiltrates; non-caseating sarcoid-like granulomas.
3. <i>Silicosis</i>	Silica dust	Lung lesions, fibrocollagenous nodules.
4. <i>Berylliosis</i>	Metallic beryllium	Sarcoid-like granulomas in lungs; fibrosis; inclusions in giant cells (asteroids, Schaumann bodies, crystals).
5. <i>Foreign body granulomas</i>	Talc, suture, oils, wood splinter etc.	Non-caseating granulomas with foreign body giant cells; demonstration of foreign body.

*Diseases discussed in this chapter.

3. *Inoculation* of the organisms into the skin may rarely occur from infected postmortem tissue.
4. *Transplacental route* results in development of congenital tuberculosis in foetus from infected mother and is a rare mode of transmission.

SPREAD OF TUBERCULOSIS. The disease spreads in the body by various routes:

1. **Local spread.** This takes place by macrophages carrying the bacilli into the surrounding tissues.
2. **Lymphatic spread.** Tuberculosis is primarily an infection of lymphoid tissues. The bacilli may pass into lymphoid follicles of pharynx, bronchi, intestines or regional lymph nodes resulting in regional tuberculous lymphadenitis which is typical of childhood infections. Primary complex is primary focus with lymphangitis and lymphadenitis.
3. **Haematogenous spread.** This occurs either as a result of tuberculous bacillaemia because of the drainage of lymphatics into the venous system or due to caseous material escaping through ulcerated wall of a vein. This produces millet seed-sized lesions in different organs of the body like lungs, liver, kidneys, bones and other tissues and is known as miliary tuberculosis.
4. **By the natural passages.** Infection may spread from:
 - i) lung lesions into pleura (tuberculous pleurisy);
 - ii) transbronchial spread into the adjacent lung segments;
 - iii) tuberculous salpingitis into peritoneal cavity (tuberculous peritonitis);
 - iv) infected sputum into larynx (tuberculous laryngitis);
 - v) swallowing of infected sputum (ileocaecal tuberculosis); and
 - vi) renal lesions into ureter and down to trigone of bladder.

HYPERSENSITIVITY AND IMMUNITY IN TUBERCULOSIS. Hypersensitivity or allergy, and immunity or resistance, play a major role in the development of lesions in tuberculosis. Tubercle bacilli as such do not produce any toxins. Tissue changes seen in tuberculosis are not the result of any exotoxin or endotoxin but are instead the result of host response to the organism which is in the form of development of cell-mediated hypersensitivity (or type IV hypersensitivity) and immunity. Both these host responses develop as a consequence of several lipids present in the micro-organism which include the following:

1. **mycosides** such as 'cord factor' which are essential for growth and virulence of the organism in the animals; and

2. **glycolipids** present in the mycobacterial cell wall like 'Wax-D' which acts as an adjuvant acting along with tuberculoprotein.

It has been known since the time of Robert Koch that the tissue reaction to tubercle bacilli is different in healthy animal not previously infected (primary infection) from an animal who is previously infected (secondary infection).

1. **In the primary infection,** intradermal injection of tubercle bacilli into the skin of a healthy guinea pig evokes no visible reaction for 10-14 days. After this period, a nodule develops at the inoculation site which subsequently ulcerates and heals poorly as the guinea pig, unlike human beings, does not possess any natural resistance. The regional lymph nodes also develop tubercles. This process is a manifestation of delayed type of hypersensitivity and is comparable to primary tuberculosis in children although healing invariably occurs in children.

2. **In the secondary infection,** the sequence of changes is different. The tubercle bacilli are injected into the skin of the guinea pig who has been infected with tuberculosis 4-6 weeks earlier. In 1-2 days, the site of inoculation is indurated and dark, attaining a diameter of about 1 cm. The skin lesion ulcerates which heals quickly and the regional lymph nodes are not affected. This is called *Koch's phenomenon* and is indicative of hypersensitivity and immunity in the host.

Similar type of changes can be produced if injection of live tubercle bacilli is replaced with old tuberculin (OT).

Hypersensitivity and immunity are closely related and are initiated through T lymphocytes sensitised against specific antigens in tuberculin. As a result of this sensitisation, lymphokines are released from T cells which induce increased microbicidal activity of the macrophages.

Tuberculin (Mantoux) skin test. This test is done by intradermal injection of 0.1 ml of tuberculoprotein, purified protein derivative (PPD). Delayed type of hypersensitivity develops in individuals who are having or have been previously infected with tuberculous infection which is identified as an indurated area of more than 15 mm in 72 hours. However, patients having disseminated tuberculosis may show negative test due to release of large amount of tuberculoproteins from the endogenous lesions masking the hypersensitivity test. A positive test is indicative of cell-mediated hypersensitivity to tubercular antigens but does not distinguish between infection and disease. The test may be false positive in atypical mycobacterial infection and

false negative in sarcoidosis, some viral infections, Hodgkin's disease and fulminant tuberculosis.

Immunisation against tuberculosis. Protective immunisation against tuberculosis is induced by injection of attenuated strains of bovine type of tubercle bacilli, *Bacille Calmette-Guérin* (BCG). Cell-mediated immunity with consequent delayed hypersensitivity reaction develops with healing of the lesion, but the cell-mediated immunity persists, rendering the host tuberculin-positive and hence immune.

EVOLUTION OF TUBERCLE. The sequence of events which take place when tubercle bacilli are introduced into the tissue are as under (Fig. 11.2):

1. When the tubercle bacilli are injected intravenously into the guinea pig, the bacilli are lodged in pulmonary capillaries where an *initial response of neutrophils* is evoked which are rapidly destroyed by the organisms.
2. After about 12 hours, there is *progressive infiltration by macrophages*. This is due to coating of tubercle bacilli with serum complement factors C_{2a} and C_{3b} which act as opsonins and attract the macrophages. Macrophages dominate the picture throughout the remaining life of the lesions. If the tubercle bacilli are, however, inhaled into the lung alveoli, macrophages predominate the picture from the beginning.
3. The macrophages start *phagocytosing* the tubercle bacilli and either kill the bacteria or die away themselves. In the latter case, they further proliferate locally as well as there is increased recruitment of macrophages from blood monocytes.
4. As a part of body's immune response, T and B cells are activated. Activated CD4+T cells develop the cell-mediated *delayed type hypersensitivity reaction*, while B cells result in formation of antibodies which play no role in body's defence against tubercle bacilli.
5. In 2-3 days, the macrophages undergo structural changes as a result of immune mechanisms—the cytoplasm becomes pale and eosinophilic and their nuclei become elongated and vesicular. These modified macrophages resemble epithelial cells and are called *epithelioid cells*.
6. The epithelioid cells in time aggregate into tight clusters or *granulomas*. Release of cytokines in response to sensitised CD4+T cells and some constituents of mycobacterial cell wall play a role in formation of granuloma.
7. Some of the macrophages form *multinucleated giant cells* by fusion of adjacent cells. The giant cells may be Langhans' type having peripherally arranged nuclei in the form of horseshoe or ring, or clustered at the two

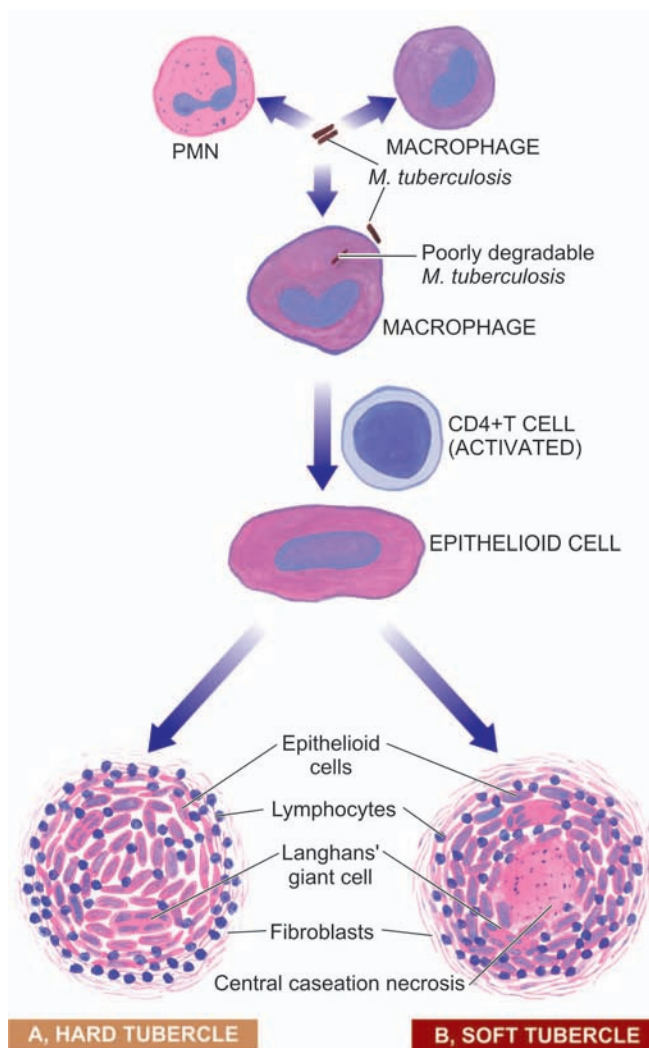


FIGURE 11.2

Schematic evolution of tubercle. In fully formed granuloma, the centre is composed of granular caseation necrosis, surrounded by epithelioid cells and Langhans' giant cells and peripheral rim of lymphocytes bounded by fibroblasts.

poles of the giant cell; or they may be foreign body type having centrally-placed nuclei.

8. Around the mass of epithelioid cells and giant cells is a zone of lymphocytes, plasma cells and fibroblasts. The lesion at this stage is called *hard tubercle* due to absence of central necrosis.

9. Within 10-14 days, the centre of the cellular mass begins to undergo caseation necrosis, characterised by cheesy appearance and high lipid content. This stage is called *soft tubercle* which is the hallmark of tuberculous lesions. The development of caseation necrosis is possibly due to interaction of mycobacteria with activated T cells (CD4+ helper T cells via IF- γ and CD8+ suppressor T cells

directly) as well as by direct toxicity of mycobacteria on macrophages. *Microscopically*, caseation necrosis is structureless, eosinophilic and granular material with nuclear debris.

10. The soft tubercle which is a fully-developed granuloma with caseous centre does not favour rapid proliferation of tubercle bacilli. *Acid-fast bacilli* are difficult to find in these lesions and may be demonstrated at the margins of recent necrotic foci and in the walls of the cavities.

The **fate of a granuloma** is variable:

- i) The caseous material may undergo liquefaction and extend into surrounding soft tissues, discharging the contents on the surface. This is called *cold abscess* although there are no pus cells in it.
- ii) In tuberculosis of tissues like bones, joints, lymph nodes and epididymis, sinuses are formed and the *sinus tracts* are lined by tuberculous granulation tissue.
- iii) The adjacent granulomas may *coalesce* together enlarging the lesion which is surrounded by progressive fibrosis.
- iv) In the granuloma enclosed by fibrous tissue, calcium salts may get deposited in the caseous material (*dystrophic calcification*) and sometimes the lesion may even get ossified over the years.

TYPES OF TUBERCULOSIS

Lung is the main organ affected in tuberculosis. Depending upon the type of tissue response and age, the infection with tubercle bacilli is of 2 main types:

- A. Primary tuberculosis; and
- B. Secondary tuberculosis.

A. Primary Tuberculosis

The infection of an individual who has not been previously infected or immunised is called *primary tuberculosis* or *Ghon's complex* or *childhood tuberculosis*.

Primary complex or Ghon's complex is the lesion produced at the portal of entry with foci in the draining lymphatic vessels and lymph nodes. The most commonly involved tissues for primary complex are lungs and hilar lymph nodes. The other tissues which may show primary complex are tonsils and cervical lymph nodes, and in the case of ingested bacilli the lesions may be found in small intestine and mesenteric lymph nodes.

The incidence of disseminated form of progressive primary tuberculosis is particularly high in immunocompromised host e.g. in patients of AIDS.

Primary complex or Ghon's complex in lungs consists of 3 components (Fig. 11.3):

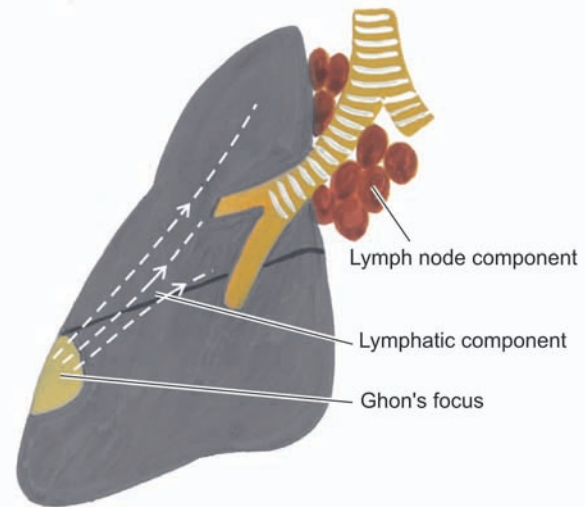


FIGURE 11.3

The primary complex composed of 3 components: Ghon's focus, draining lymphatics, and hilar lymph nodes.

1. Pulmonary component. Lesion in the lung is the primary focus or Ghon's focus. It is 1-2 cm solitary area of tuberculous pneumonia located peripherally under a patch of pleurisy, in any part of the lung but more often in subpleural focus in the upper part of lower lobe.

Microscopically, the lung lesion consists of tuberculous granulomas with caseation necrosis.

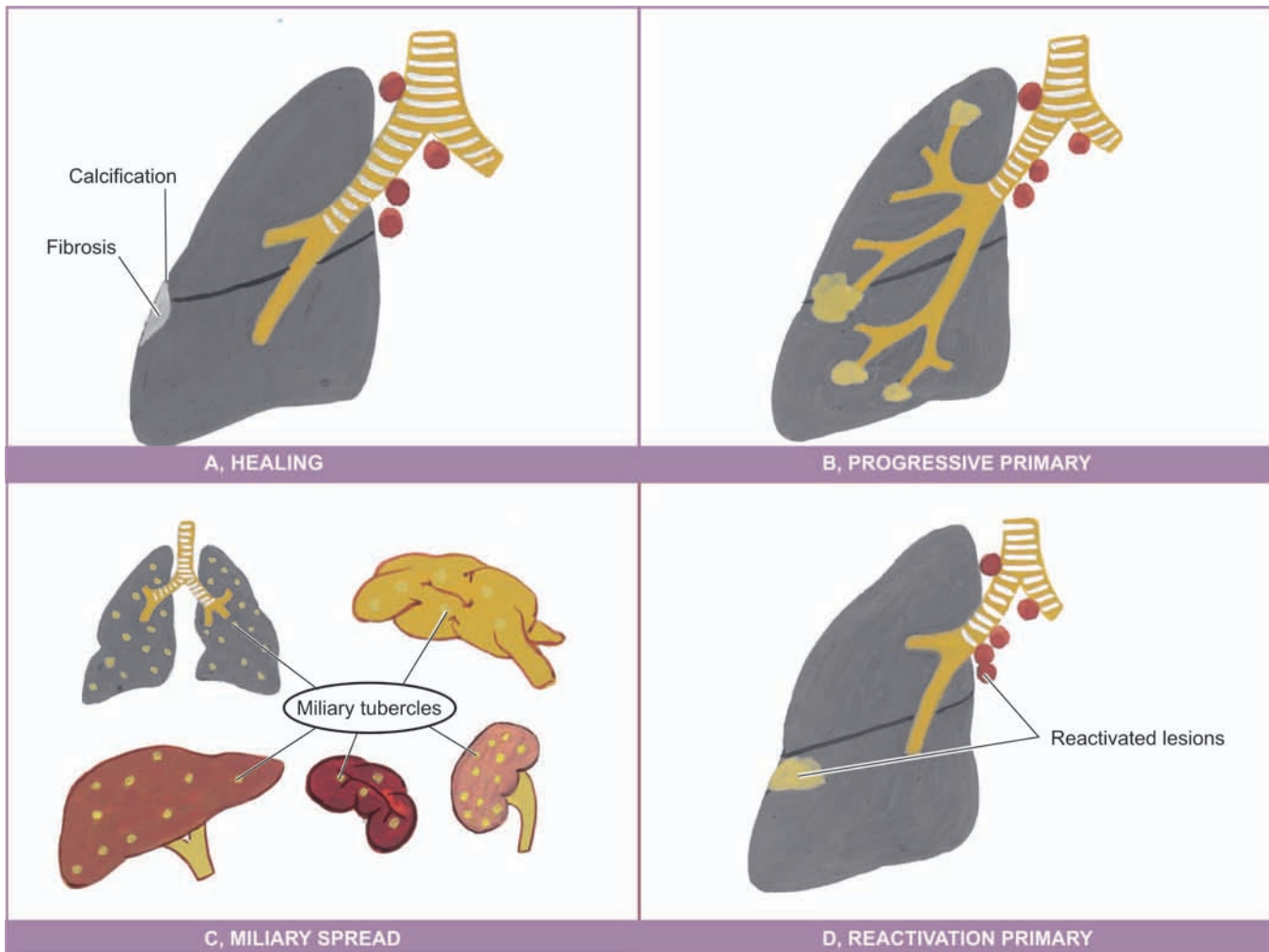
2. Lymphatic vessel component. The lymphatics draining the lung lesion contain phagocytes containing bacilli and may develop beaded, miliary tubercles along the path of hilar lymph nodes.

3. Lymph node component. This consists of enlarged hilar and tracheo-bronchial lymph nodes in the area drained. The affected lymph nodes are matted and show caseation necrosis.

Microscopically, the lesions are characterised by extensive caseation, tuberculous granulomas and fibrosis. Nodal lesions are potential source of re-infection later (COLOUR PLATE IV: CL 15).

In the case of primary tuberculosis of alimentary tract due to ingestion of tubercle bacilli, a small primary focus is seen in the intestine with enlarged mesenteric lymph nodes producing *tabes mesenterica*. The enlarged and caseous mesenteric lymph nodes may rupture into peritoneal cavity and cause tuberculous peritonitis.

FATE OF PRIMARY TUBERCULOSIS. Primary complex may have one of the following sequelae (Fig. 11.4):

**FIGURE 11.4**

Sequelae of primary complex. A, Healing by fibrosis and calcification. B, Progressive primary tuberculosis spreading to the other areas of the same lung or opposite lung. C, Miliary spread to lungs, liver, spleen, kidneys and brain. D, Progressive secondary pulmonary tuberculosis from reactivation of dormant primary complex.

1. The lesions of primary tuberculosis of lung commonly do not progress but instead heal by *fibrosis*, and in time undergo *calcification* and even *ossification*.
2. In some cases, the primary focus in the lung continues to grow and the caseous material is disseminated through bronchi to the other parts of the same lung or the opposite lung. This is called *progressive primary tuberculosis*.
3. At times, bacilli may enter the circulation through erosion in a blood vessel and spread to various tissues and organs. This is called *primary miliary tuberculosis* and the lesions are seen in organs like liver, spleen, kidney, brain and bone marrow.
4. In certain circumstances like in lowered resistance and increased hypersensitivity of the host, the healed

lesions of primary tuberculosis may get reactivated. The bacilli lying dormant in acellular caseous material are activated and cause *progressive secondary tuberculosis*. It affects children more commonly but adults may also develop this kind of progression.

B. Secondary Tuberculosis

The infection of an individual who has been previously infected or sensitised is called *secondary*, or *post-primary* or *reinfection*, or *chronic tuberculosis*.

The infection may be acquired from (Fig. 11.5):

- *endogenous source* such as reactivation of dormant primary complex; or
- *exogenous source* such as fresh dose of reinfection by the tubercle bacilli.



FIGURE 11.5

Progressive secondary tuberculosis. A, Endogenous infection from reactivation of dormant primary complex. B, Exogenous infection from fresh dose of tubercle bacilli.

Secondary tuberculosis occurs most commonly in lungs in the region of apex. Other sites and tissues which can be involved are tonsils, pharynx, larynx, small intestine and skin. Secondary tuberculosis of other organs and tissues is described in relevant chapters later while that of lungs is discussed here.

Secondary Pulmonary Tuberculosis

The lesions in secondary pulmonary tuberculosis usually begin as 1-2 cm apical area of consolidation of the lung, which may in time develop a small area of central caseation necrosis and peripheral fibrosis. It occurs by haematogenous spread of infection from primary complex to the apex of the affected lung where the oxygen tension is high and favourable for growth of aerobic tubercle bacilli. *Microscopically*, the appearance is typical of tuberculous granulomas with caseation necrosis.

Patients with HIV infection previously exposed to tuberculous infection have particularly high incidence of reactivation of primary tuberculosis and the pattern of lesions in such cases is similar to that of primary tuberculosis i.e. with involvement of hilar lymph nodes rather than cavitory and apical lesions in the lung. In addition, opportunistic infection with *M. avium-intracellulare* can occur in cases of AIDS.

FATE OF SECONDARY PULMONARY TUBERCULOSIS. The subapical lesions in lungs can have the following courses:

1. The lesions may *heal* with fibrous scarring and calcification.
2. The lesions may *coalesce* together to form larger area of tuberculous pneumonia and produce progressive secondary pulmonary tuberculosis with the following pulmonary and extrapulmonary involvements:
 - i) Fibrocaceous tuberculosis
 - ii) Tuberculous caseous pneumonia
 - iii) Miliary tuberculosis.

I. FIBROCACEOUS TUBERCULOSIS. The original area of tuberculous pneumonia undergoes massive central caseation necrosis which may:

- either break into a bronchus from a cavity (*cavitory or open fibrocaceous tuberculosis*); or
- remain, as a soft caseous lesion without drainage into a bronchus or bronchiole to produce a non-cavitory lesion (*chronic fibrocaceous tuberculosis*).

The cavity provides favourable environment for proliferation of tubercle bacilli due to high oxygen tension. The cavity may communicate with bronchial tree and becomes the source of spread of infection ('*open tuberculosis*'). The open case of secondary tuberculosis may implant tuberculous lesion on the mucosal lining of air passages producing *endobronchial and endotracheal tuberculosis*. Ingestion of sputum containing tubercle bacilli from endogenous pulmonary lesions may produce *laryngeal and intestinal tuberculosis*.

Grossly, tuberculous cavity is spherical with thick fibrous wall, lined by yellowish, caseous, necrotic material and the lumen is traversed by thrombosed blood vessels. Around the wall of cavity are seen foci of consolidation. The overlying pleura may also be thickened (Fig. 11.6,A)

Microscopically, the wall of cavity shows eosinophilic, granular, caseous material which may show foci of dystrophic calcification. Widespread coalesced tuberculous granulomas composed of epithelioid cells, Langhans' giant cells and peripheral mantle of lymphocytes and having central caseation necrosis are seen. The outer wall of cavity shows fibrosis (Fig. 11.6,B) (COLOUR PLATE IV: CL 14).

Complications of cavitory secondary tuberculosis are:

- aneurysms of patent arteries crossing the cavity producing haemoptysis;
- extension to pleura producing bronchopleural fistula;
- tuberculous empyema from deposition of caseous material on the pleural surface; and
- thickened pleura from adhesions of parietal pleura.

II. TUBERCULOUS CASEOUS PNEUMONIA. The caseous material from a case of secondary tuberculosis in an individual with high degree of hypersensitivity may spread to rest of the lung producing caseous pneumonia (Fig. 11.7,A).

Microscopically, the lesions show exudative reaction with oedema, fibrin, polymorphs and monocytes but numerous tubercle bacilli can be demonstrated in the exudates (Fig. 11.7,B).

III. MILIARY TUBERCULOSIS. This is lymphohaematogenous spread of tuberculous infection from primary focus or later stages of tuberculosis. The spread may occur to systemic organs or isolated organ. The spread is either by entry of infection into pulmonary vein producing disseminated or isolated organ lesion in different extra-pulmonary sites (e.g. liver, spleen, kidney, brain and bone marrow) or into pulmonary artery restricting the development of miliary lesions within the lung (Fig. 11.8,A). The miliary lesions are millet seed-sized (1 mm diameter), yellowish, firm areas without grossly visible caseation necrosis.

Microscopically, the lesions show the structure of tubercles with minute areas of caseation necrosis (Fig. 11.8,B).

Clinical Features of Tuberculosis

The clinical manifestations in tuberculosis may be variable depending upon the location, extent and type of lesions. However, in secondary pulmonary tuberculosis which is the common type, the usual clinical features are as under:

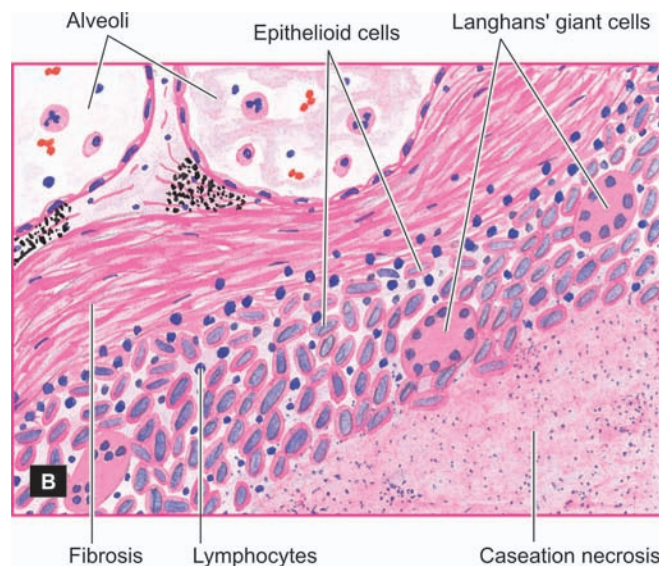
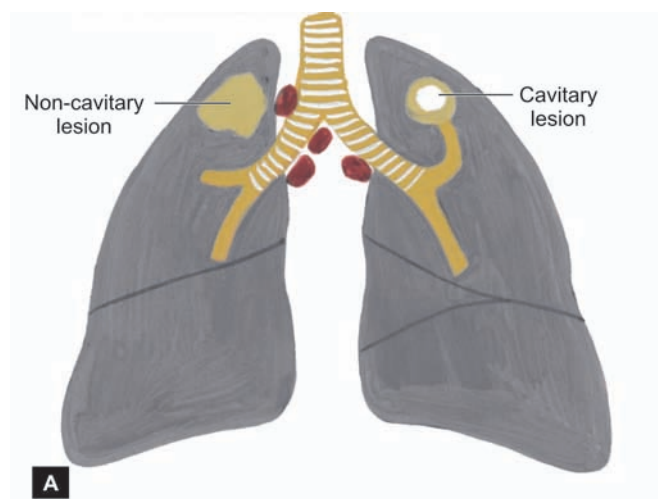


FIGURE 11.6

Fibrocaceous tuberculosis. A, Non-cavitary (chronic) fibrocaceous tuberculosis (left) and cavitary/open fibrocaceous tuberculosis (right). B, Microscopic appearance of lesions of secondary fibrocaceous tuberculosis of the lung showing wall of the cavity.

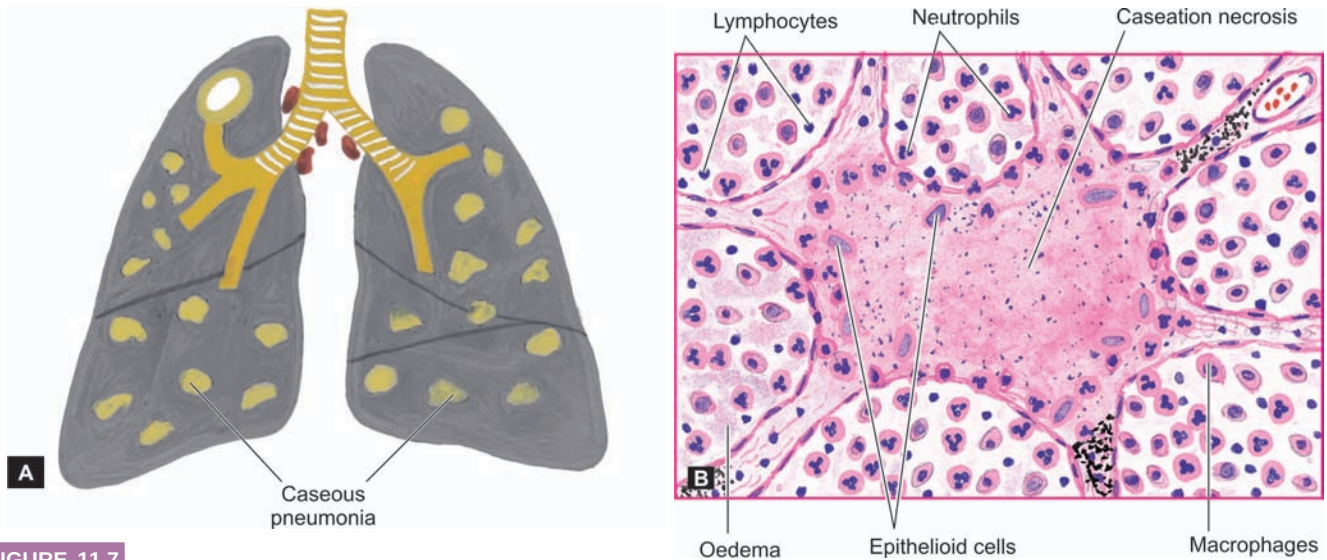


FIGURE 11.7

A, Bilateral tuberculous caseous pneumonia. B, Tuberculous caseous pneumonia showing exudative reaction. In AFB staining, these cases have numerous acid-fast bacilli (not shown here).

1. Referable to lungs—such as productive cough, may be with haemoptysis, pleural effusion, dyspnoea, orthopnoea etc. Chest X-ray may show typical apical changes like pleural effusion, nodularity, and miliary or diffuse infiltrates in the lung parenchyma.

2. Systemic features—such as fever, night sweats, fatigue, loss of weight and appetite. Long-standing and untreated cases of tuberculosis may develop systemic secondary amyloidosis.

Causes of death in pulmonary tuberculosis are usually pulmonary insufficiency, pulmonary haemorrhage, sepsis due to disseminated miliary tuberculosis, cor pulmonale or secondary amyloidosis.

Intestinal Tuberculosis

Intestinal tuberculosis can occur in 3 forms—primary, secondary and hyperplastic caecal tuberculosis.

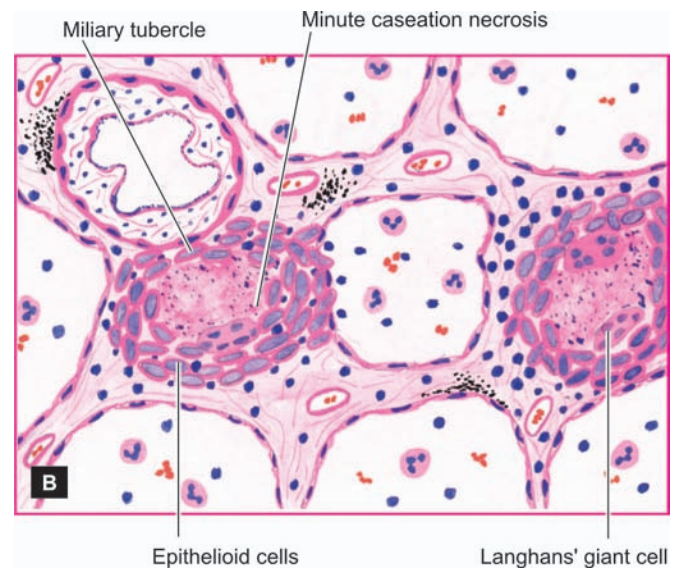
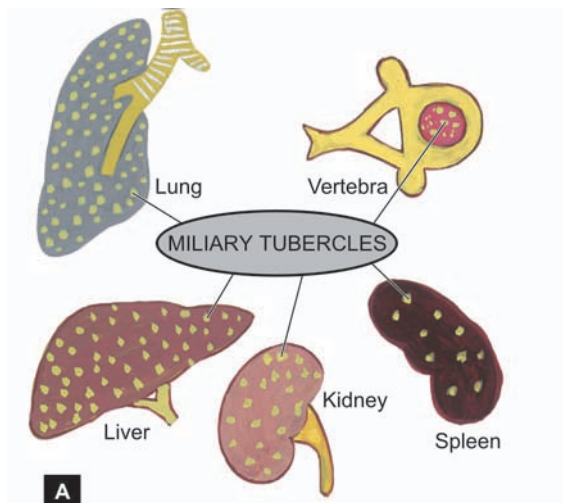


FIGURE 11.8

A, Acute miliary tuberculosis affecting different organs (lung, liver, spleen, kidney and vertebra) B, Miliary tubercles in lung having minute central caseation necrosis.

1. PRIMARY INTESTINAL TUBERCULOSIS. Though an uncommon disease in the Western world, primary tuberculosis of the ileocaecal region is quite common in India. It used to occur by ingestion of unpasteurised cow's milk infected with *Mycobacterium bovis*. But now-a-days due to control of tuberculosis in cattle and pasteurisation of milk, virtually all cases of intestinal tuberculosis are caused by *M. tuberculosis*. The predominant changes are in the mesenteric lymph nodes without any significant intestinal lesion.

Grossly, the affected lymph nodes are enlarged, matted and caseous (tabes mesenterica). Eventually, there is healing by fibrosis and calcification (Fig. 11.9,A).

Microscopically, in the initial stage, there is primary complex or Ghon's focus in the intestinal mucosa as occurs elsewhere in primary tuberculous infection (Page 121). Subsequently, the mesenteric lymph nodes are affected which show typical tuberculous granulomatous inflammatory reaction with caseation necrosis. Tuberculous peritonitis may occur due to spread of the infection.

2. SECONDARY INTESTINAL TUBERCULOSIS. Swallowing of sputum in patients with active pulmonary tuberculosis may cause secondary intestinal tuberculosis, most commonly in the terminal ileum and rarely in the colon.

Grossly, the intestinal lesions are prominent than the lesions in regional lymph nodes as in secondary

pulmonary tuberculosis. The lesions begin in the Peyer's patches or the lymphoid follicles with formation of small ulcers that spread through the lymphatics to form large ulcers which are *transverse to the long axis of the bowel*, (c.f. typhoid ulcers of small intestine, described below). These ulcers may be coated with caseous material. Serosa may be studded with visible tubercles. In advanced cases, transverse fibrous strictures and intestinal obstruction are seen (Fig. 11.9,B).

Microscopically, the tuberculous lesions in the intestine are similar to those observed elsewhere i.e. presence of tubercles. Mucosa and submucosa show ulceration and the muscularis may be replaced by variable degree of fibrosis. Tuberculous peritonitis may be observed.

3. HYPERPLASTIC CAECAL TUBERCULOSIS. This is a variant of secondary tuberculosis secondary to pulmonary tuberculosis.

Grossly, the caecum and/or ascending colon are thick-walled with mucosal ulceration. Clinically, the lesion is palpable and may be mistaken for carcinoma (Fig. 11.9,C).

Microscopically, the presence of caseating tubercles distinguishes the condition from Crohn's disease in which granulomas are non-caseating. Besides, bacteriological evidence by culture or animal inoculation and Mantoux test are helpful in differential diagnosis of the two conditions.

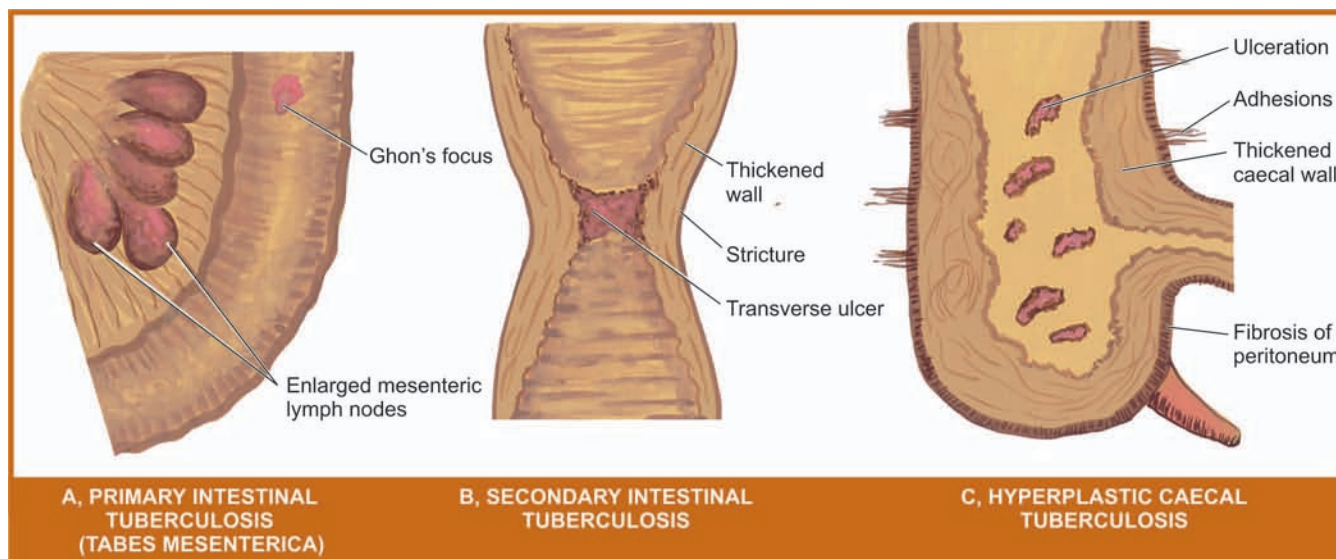


FIGURE 11.9

Intestinal tuberculosis, three patterns.

LEPROSY

Leprosy or Hansen's disease (after discovery of the causative organism by Hansen in 1874) is a chronic infectious disease affecting mainly the cooler parts of the body such as the skin, mouth, respiratory tract, eyes, peripheral nerves, superficial lymph nodes and testis. Though the earliest and main involvement in leprosy is of the skin and nerves but in bacteraemia from endothelial colonisation or by bacilli filtered from blood by reticuloendothelial system, other organs such as the liver, spleen, bone marrow and regional lymph nodes are also involved. Advanced cases may develop secondary amyloidosis and renal disease, both of which are of immunologic origin.

Causative Organism

The disease is caused by *Mycobacterium leprae* which closely resembles *Mycobacterium tuberculosis* but is less acid-fast. The organisms in tissues appear as compact rounded masses (*globi*) or are arranged in parallel fashion like *cigarettes-in-pack*.

M. leprae can be demonstrated in tissue sections, in split skin smears by splitting the skin, scrapings from cut edges of dermis, and in nasal smears by the following techniques:

1. *Acid-fast (Ziehl-Neelsen) staining*. The staining procedure is similar as for demonstration of *M. tuberculosis* but can be decolourised by lower concentration (5%) of sulphuric acid (less acid-fast).
2. *Fite-Faraco staining* procedure is a modification of Z.N. procedure and is considered better for more adequate staining of tissue sections.
3. *Gomori methenamine silver (GMS) staining* can also be employed.

The slit smear technique gives a reasonable quantitative measure of *M. leprae* when stained with Ziehl-Neelsen method and examined under 100x oil objective for determining the density of bacteria in the lesion (*bacterial index, BI*). B.I. is scored from 1+ to 6+ (range from 1 to 10 bacilli per 100 fields to > 1000 per field) as *multibacillary* leprosy while B.I. 0+ is termed *paucibacillary*.

Nine-banded armadillo, a rodent, acts as an experimental animal model as it develops leprosy which is histopathologically and immunologically similar to human leprosy.

Incidence

The disease is endemic in areas with hot and moist climates and in poor tropical countries. According to the WHO, five countries—India, Brazil, Indonesia,

Myanmar (Burma) and Nigeria, together constitute vast majority of leprosy cases, of which India accounts for about one-third of all registered leprosy cases globally. In India, the disease is seen more commonly in regions like Tamil Nadu, Bihar, Pondicherry, Andhra Pradesh, Orissa, West Bengal and Assam. Very few cases are now seen in Europe and the United States.

Mode of Transmission

Leprosy is a slow communicable disease and the incubation period between first exposure and appearance of signs of disease varies from 2 to 20 years (average about 3 years). The infectivity may be from the following sources:

1. *Direct contact* with untreated leprosy patients who shed numerous bacilli from damaged skin, nasal secretions, mucous membrane of mouth and hair follicles.
2. *Materno-foetal transmission* across the placenta.
3. Transmission from *milk* of leprosy patient to infant.

Classification

Leprosy is broadly classified into 2 main types:

- Lepromatous type representing *low resistance*; and
- Tuberculoid type representing *high resistance*.

Salient differences between the two main forms of leprosy are summarised in Table 11.2.

Since both these types of leprosy represent two opposite poles of host immune response, these are also called *polar forms* of leprosy. Cases not falling into either of the two poles are classified as *borderline* and *indeterminate types*.

Currently, leprosy is classified into 7 groups (*modified Ridley and Jopling's classification*). These are:

- TT—Tuberculoid Polar (*High resistance*)
- BT—Borderline Tuberculoid
- TI—Tuberculoid Indefinite
- BB—Mid Borderline
- LI—Lepromatous Indefinite
- BL—Borderline Lepromatous
- LL—Lepromatous Polar (*Low resistance*)

In addition, not included in Ridley-Jopling's classification are cases of indeterminate leprosy, pure neural leprosy and histoid leprosy resembling a nodule of dermatofibroma and positive for lepra bacilli.

Reactions in Leprosy

There may be two types of lepra reactions: type I (borderline reactions), and type II (erythema nodosum leprosum).

TABLE 11.2: Differences between Lepromatous and Tuberculoid Leprosy.

FEATURE	LEPROMATOUS LEPROSY	TUBERCULOID LEPROSY
1. <i>Skin lesions</i>	Symmetrical, multiple, hypopigmented, erythematous, maculopapular or nodular (leonine facies).	Asymmetrical, single or a few lesions, hypopigmented and erythematous macular.
2. <i>Nerve involvement</i>	Present but sensory disturbance is less severe.	Present with distinct sensory disturbance.
3. <i>Histopathology</i>	Collection of foamy macrophages or lepra cells in the dermis separated from epidermis by a 'clear zone'.	Hard tubercle similar to granulomatous lesion, eroding the basal layer of epidermis; no clear zone.
4. <i>Bacteriology</i>	Lepra cells highly positive for lepra bacilli seen as 'globi' or 'cigarettes-in-pack' appearance.	Lepra bacilli few, seen in destroyed nerves as granular or beaded forms.
5. <i>Immunity</i>	Suppressed (low resistance).	Good immune response (high resistance).
6. <i>Lepromin test</i>	Negative.	Positive.

TYPE I: BORDERLINE REACTIONS. The polar forms of leprosy do not undergo any change in clinical and histopathological picture. The borderline groups are unstable and may move across the spectrum in either direction with upgrading or downgrading of patient's immune state. Accordingly, there may be two types of borderline reaction:

1. Upgrading reaction is characterised by increased cell-mediated immunity and occurs in patients of borderline lepromatous (BL) type on treatment who upgrade or shift towards tuberculoid type.

Microscopically, the upgrading reaction shows an increase of lymphocytes, oedema of the lesions and reduced B.I.

2. Downgrading reaction is characterised by lowering of cellular immunity and is seen in borderline tuberculoid (BT) type who downgrade or shift towards lepromatous type.

Microscopically, the lesions show dispersal and spread of the granulomas and increased presence of lepra bacilli.

TYPE II: ERYTHEMA NODOSUM LEPROSUM (ENL). ENL occurs in lepromatous patients after treatment. It is characterised by tender cutaneous nodules, fever, iridocyclitis, synovitis and lymph node involvement.

Microscopically, the lesions in ENL show infiltration by neutrophils and prominence of vasculitis. Secondary amyloidosis may follow repeated attacks of ENL in leprosy.

Clinical Features

The two main forms of leprosy show distinctive clinical features:

1. Lepromatous Leprosy:

i) The skin lesions in LL are generally symmetrical, multiple, slightly hypopigmented and erythematous macules, papules, nodules or diffuse infiltrates. The nodular lesions may coalesce to give *leonine facies* appearance.

ii) The lesions are hypoaesthetic or anaesthetic but the sensory disturbance is not as distinct as in TT.

2. Tuberculoid leprosy:

i) The skin lesions in TT occur as either single or as a few asymmetrical lesions which are hypopigmented and erythematous macules.

ii) There is a distinct sensory impairment.

Histopathology of Leprosy

Usually, skin biopsy from the margin of lesions is submitted for diagnosis and for classification of leprosy. The histopathologic diagnosis of multibacillary leprosy like LL and BL offers no problem while the indeterminate leprosy and tuberculoid lesions are paucibacillary and their diagnosis is made together with clinical evidence.

In general, for histopathologic evaluation in all suspected cases of leprosy the following broad *guidelines* should be followed:

- cell type of granuloma;
- nerve involvement; and
- bacterial load.

The main features in various groups are given below.

1. Lepromatous leprosy:

The following features characterise lepromatous polar leprosy (Fig. 11.10,A) (COLOUR PLATE IV: CL 16):

- In the dermis, there is proliferation of macrophages with foamy change, particularly around the blood vessels, nerves and dermal appendages. The foamy macrophages are called '*lepra cells*' or *Virchow cells*.
- The lepra cells are heavily laden with acid-fast bacilli demonstrated with AFB staining. The AFB may be seen as compact globular masses (*globi*) or arranged in parallel fashion like '*cigarettes-in-pack*' (COLOUR PLATE IV: CL 16, inbox).
- The dermal infiltrate of lepra cells characteristically does not encroach upon the basal layer of epidermis and is separated from epidermis by a subepidermal uninvolved *clear zone*.
- The *epidermis* overlying the lesions is thinned out, flat and may even ulcerate

2. Tuberculoid leprosy:

The polar tuberculoid form presents the following histological features (Fig. 11.10,B):

- The dermal lesions show granulomas resembling *hard tubercles* composed of epithelioid cells, Langhans' giant cells and peripheral mantle of lymphocytes.
- Lesions of tuberculoid leprosy have predilection for *dermal nerves* which may be destroyed and infiltrated by epithelioid cells and lymphocytes.
- The granulomatous infiltrate erodes the basal layer of epidermis i.e. there is *no clear zone*.
- The *lepra bacilli* are few and seen in destroyed nerves.

3. Borderline leprosy:

The histopathologic features of the three forms of borderline leprosy are as under:

- Borderline tuberculoid (BT)* form shows epithelioid cells and plentiful lymphocytes. There is a narrow clear subepidermal zone. Lepra bacilli are scanty and found in nerves.
- Borderline lepromatous (BL)* form shows predominance of histiocytes, a few epithelioid cells and some irregularly dispersed lymphocytes. Numerous lepra bacilli are seen.
- Mid-borderline (BB)* form shows sheets of epithelioid cells with no giant cells. Some lymphocytes are seen in the peri-neurium. Lepra bacilli are present, mostly in nerves.

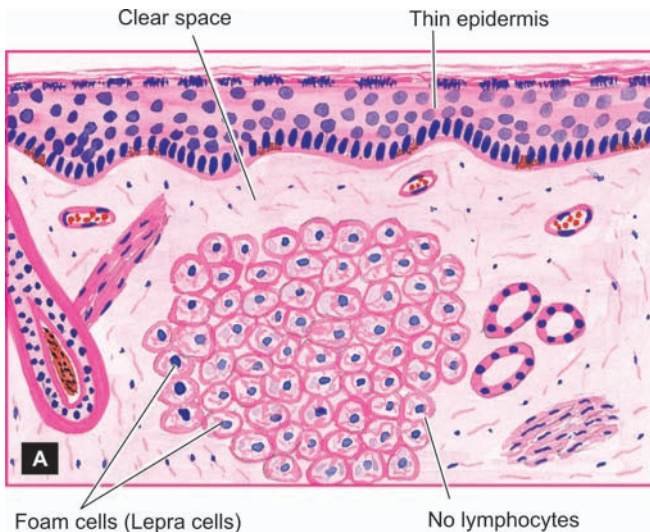
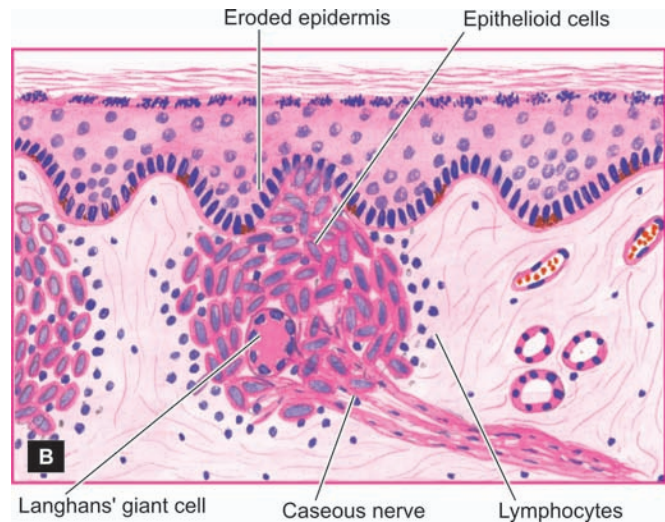


FIGURE 11.10

Polar forms of leprosy.

A, *Lepromatous leprosy (LL)*. Proliferation of lepra cells around blood vessels and dermal adnexal structures with subepidermal clear zone. These cells show '*Globi*' and '*cigarettes-in-pack*' appearance of bacilli in AFB staining (not shown here).



B, *Tuberculoid leprosy (TT)*. Granulomatous lesion in the vicinity of dermal nerve without clear subepidermal zone. The granuloma would show scanty and granular forms of bacilli in a destroyed nerve in AFB staining (not shown here).

4. Indeterminate leprosy:

The histopathologic features are non-specific so that the diagnosis of non-specific chronic dermatitis may be made. However, a few features help in suspecting leprosy as under:

- i) Lymphocytic or mononuclear cell infiltrate, focalised particularly around skin adnexal structures like hair follicles and sweat glands or around blood vessels.
- ii) Nerve involvement, if present, is strongly supportive of diagnosis.
- iii) Confirmation of diagnosis is made by finding of lepra bacilli.

Immunology of Leprosy

Lepromin test is not a diagnostic test but is used for classifying leprosy on the basis of immune response. Intradermal injection of lepromin, an antigenic extract of *M. leprae*, reveals delayed hypersensitivity reaction in patients of tuberculoid leprosy.

- An early positive reaction appearing as an indurated area in 24-48 hours is called *Fernandez reaction*.
- A delayed granulomatous lesion appearing after 3-4 weeks is called *Mitsuda reaction*.

Patients of lepromatous leprosy are negative by the lepromin test.

The test indicates that cell-mediated immunity is greatly suppressed in LL while patients of TT show good immune response. Delayed type of hypersensitivity is conferred by T helper cells. The granulomas of TT have sufficient T helper cells and fewer T suppressor cells at the periphery while the cellular infiltrates of LL lack T helper cells.

Though the patients of LL have humoral components like high levels of immunoglobulins (IgG, IgA, IgM) and antibodies to mycobacterial antigens but these antibodies do not have any protective role.

Anti-leprosy vaccines are now being developed and undergoing human trials but since the incubation period of leprosy is quite long, the efficacy of such vaccines will be known after a number of years.

SYPHILIS

Syphilis is a venereal (sexually-transmitted) disease caused by spirochaetes, *Treponema pallidum*. Other treponemal diseases are yaws, pinta and bejel. The word 'syphilis' is derived from the name of the mythological handsome boy, Syphilus, who was cursed by Apollo with the disease.

Causative Organism

T. pallidum is a coiled spiral filament 10 µm long that moves actively in fresh preparations. The organism cannot be stained by the usual methods and can be demonstrated in the exudates and tissues by:

1. *dark ground illumination (DGI)* in fresh preparation;
2. *fluorescent antibody technique*; and
3. *silver impregnation techniques*.

The organism has not been cultivated in any culture media but experimental infection can be produced in rabbits and chimpanzees. The organism is rapidly destroyed by cold, heat, and antiseptics.

Immunology

T. pallidum does not produce any endotoxin or exotoxin. The pathogenesis of the lesions appears to be due to host immune response.

Treponemal infection is associated with two important antibodies which are immunoglobulins. These are as under:

1. **The Wassermann antibodies.** Wassermann described a complement fixing antibody against antigen of human syphilitic tissue. This antigen is used in the Standard Test for Syphilis (STS) as follows:
 - i) Wassermann complement fixing test; and
 - ii) Venereal Disease Research Laboratory (VDRL) test.
2. **Treponemal antibodies.** Treponemal antibodies are produced which react with treponemal protein. The tests employed for detecting these antibodies are:
 - i) Reiter protein complement fixation (RPCF) test;
 - ii) Treponema pallidum immobilisation (TPI) test;
 - iii) Fluorescent treponemal antibody (FTA) test; and
 - iv) Treponemal passive haemagglutination (TPHA) test.

Mode of Transmission

Syphilitic infection can be transmitted by the following routes:

1. *Sexual intercourse* resulting in lesions on glans penis, vulva, vagina and cervix.
2. *Intimate person-to-person contact* with lesions on lips, tongue or fingers.
3. *Transfusion* of infected blood.
4. *Materno-foetal transmission* in congenital syphilis if the mother is infected.

Stages of Acquired Syphilis

Acquired syphilis is divided into 3 stages depending upon the period after which the lesions appear and the

type of lesions. These are: primary, secondary and tertiary syphilis.

1. PRIMARY SYPHILIS. Typical lesion of primary syphilis is *chancre* which appears on genitals or at extra-genital sites in 2-4 weeks after exposure to infection (Fig. 11.11,A). Initially, the lesion is a painless papule which ulcerates in the centre so that the fully-developed chancre is an indurated lesion with central ulceration accompanied by regional lymphadenitis. The chancre heals without scarring, even in the absence of treatment.

Microscopically, the chancre is characterised by:

- i) Dense infiltrate of lymphocytes, plasma cells and a few macrophages.
- ii) Perivascular aggregation of mononuclear cells, particularly plasma cells (periarteritis and endarteritis).

Antibody tests are positive in 1-3 weeks after the appearance of chancre. Spirochaetes can be demonstrated in the exudates by DGI.

2. SECONDARY SYPHILIS. Inadequately treated patients of primary syphilis develop *mucocutaneous lesions* and painless lymphadenopathy in 2-3 months after the exposure (Fig. 11.11,B). Mucocutaneous lesions may

be in the form of the mucous patches on mouth, pharynx and vagina; and generalised skin eruptions and condyloma lata in anogenital region.

Antibody tests are always positive at this stage. Secondary syphilis is *highly infective stage* and spirochaetes can be easily demonstrated in the mucocutaneous lesions.

3. TERTIARY SYPHILIS. After a latent period of appearance of secondary lesions and about 2-3 years following first exposure, tertiary lesions of syphilis appear. Lesions of tertiary syphilis are much less infective than the other two stages and spirochaetes can be demonstrated with great difficulty. These lesions are of 2 main types (Fig. 11.11,C):

i) **Syphilitic gumma.** It is a solitary, localised, rubbery lesion with central necrosis, seen in organs like liver, testis, bone and brain. In liver, the gumma is associated with scarring of hepatic parenchyma (*hepar lobatum*).

Microscopically, the structure of gumma shows (Fig. 11.12):

- a) central coagulative necrosis resembling caseation but is less destructive so that outlines of necrosed cells can still be faintly seen; and
- b) surrounding zone of palisaded macrophages with lymphocytes, plasma cells, giant cells and fibroblasts.

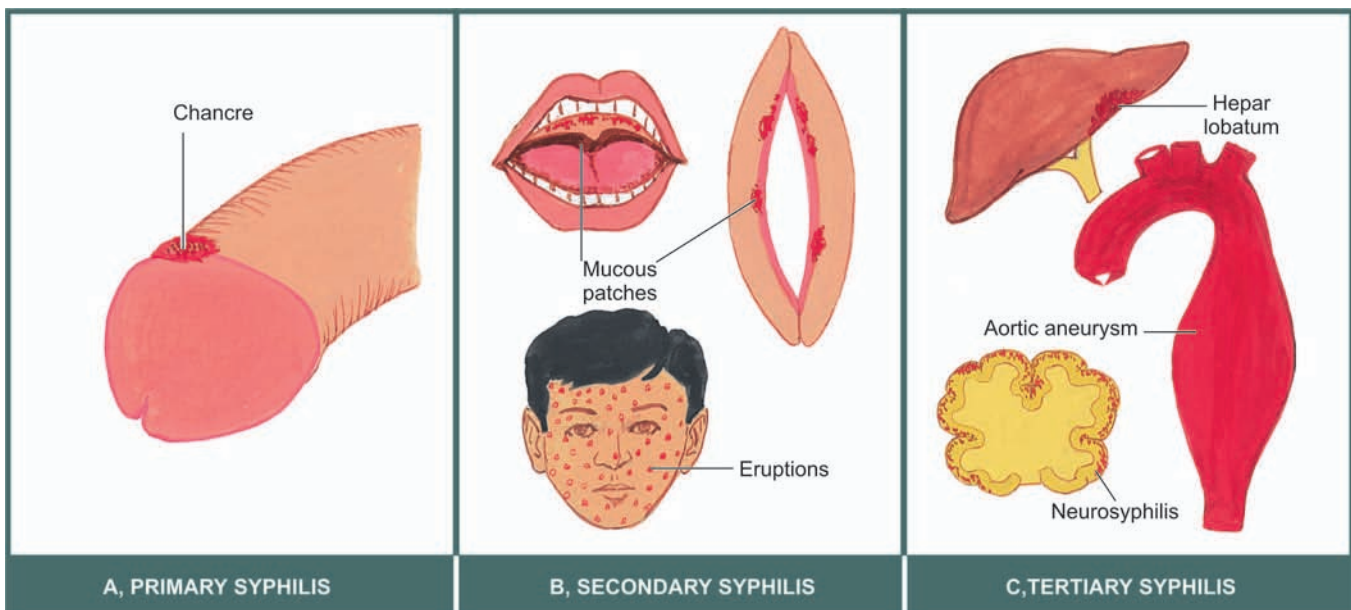


FIGURE 11.11

Organ involvement in various stages of acquired syphilis.

A, *Primary syphilis*: Primary lesion is 'chancre' on glans penis. B, *Secondary syphilis*: Mucocutaneous lesions—mucous patches on oral and vaginal mucosa and generalised skin eruptions. C, *Tertiary syphilis*: Localised lesion as gumma of liver with scarring (*hepar lobatum*); diffuse lesions (right) in aorta (aneurysm, narrowing of mouths of coronary ostia and incompetence of aortic valve ring) and nervous system.

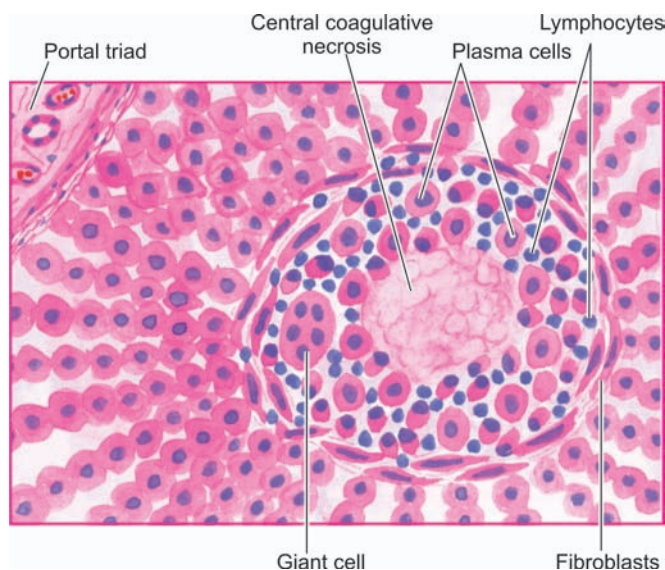


FIGURE 11.12

Typical microscopic appearance in the case of syphilitic gumma of the liver. Central coagulative necrosis is surrounded by palisades of macrophages and plasma cells margined peripherally by fibroblasts.

ii) Diffuse lesions of tertiary syphilis. The lesions appear following widespread dissemination of spirochaetes in the body. The diffuse lesions are predominantly seen in cardiovascular and nervous systems which are described in detail later in the relevant chapters. Briefly, these lesions are as under:

- a) *Cardiovascular syphilis* mainly involves thoracic aorta. The wall of aorta is weakened and dilated due to syphilitic aortitis and results in aortic aneurysm, incompetence of aortic valve and narrowing of mouths of coronary ostia.
- b) *Neurosyphilis* may manifest as:
 - meningovascular syphilis affecting chiefly the meninges;
 - tabes dorsalis affecting the spinal cord; and
 - general paresis affecting the brain.

CONGENITAL SYPHILIS. Congenital syphilis may develop in a foetus of more than 16 weeks gestation who is exposed to maternal spirochaetaemia. There can be 3 possibilities:

1. **The child born dead.** The child is premature, with macerated skin, enlarged spleen and liver, and with syphilitic epiphysitis.
2. **The child born alive (Infantile form).** The child may show mucocutaneous lesions of acquired second-

dary syphilis and bony lesions like epiphysitis and periostitis. The bridge of nose may fall due to ulceration and destruction giving the characteristic 'saddle nose' appearance of congenital syphilis.

3. **The late type.** The lesions appear after some years. The characteristic 'Hutchinson's teeth' seen in this type are small, widely spaced, peg-shaped permanent teeth. Tertiary lesions like gummas and neurosyphilis may also be seen.

Microscopically, mononuclear cell infiltration is seen in most internal organs in congenital syphilis. Many spirochaetes can be demonstrated in involved tissues.

ACTINOMYCOSIS

Actinomycosis is a chronic suppurative disease caused by anaerobic bacteria, *Actinomyces israelii*. The disease is conventionally included in mycology though the causative organism is filamentous bacteria and not true fungus. The disease is worldwide in distribution. The organisms are commensals in the oral cavity, alimentary tract and vagina. The infection is always endogeneous in origin and not person-to-person. The organisms invade, proliferate and disseminate in favourable conditions like break in mucocutaneous continuity, some underlying disease etc.

PATHOLOGIC CHANGES. Depending upon the anatomic location of lesions, actinomycosis is of 4 types: cervicofacial, thoracic, abdominal, and pelvic (Fig. 11.13,A).

1. **Cervicofacial actinomycosis.** This is the commonest form (60%) and has the best prognosis. The infection enters from tonsils, carious teeth, periodontal disease or trauma following tooth extraction. Initially, a firm swelling develops in the lower jaw ('lumpy jaw'). In time, the mass breaks down and abscesses and sinuses are formed. The discharging pus contains typical tiny yellow sulphur granules. The infection may extend into adjoining soft tissues as well as may destroy the bone.

2. **Thoracic actinomycosis.** The infection in the lungs is due to aspiration of the organism from oral cavity or extension of infection from abdominal or hepatic lesions. Initially, the disease resembles pneumonia but subsequently the infection spreads to the whole of lung, pleura, ribs and vertebrae.

3. **Abdominal actinomycosis.** This type is common in appendix, caecum and liver. The abdominal infection results from swallowing of organisms from oral cavity or extension from thoracic cavity.

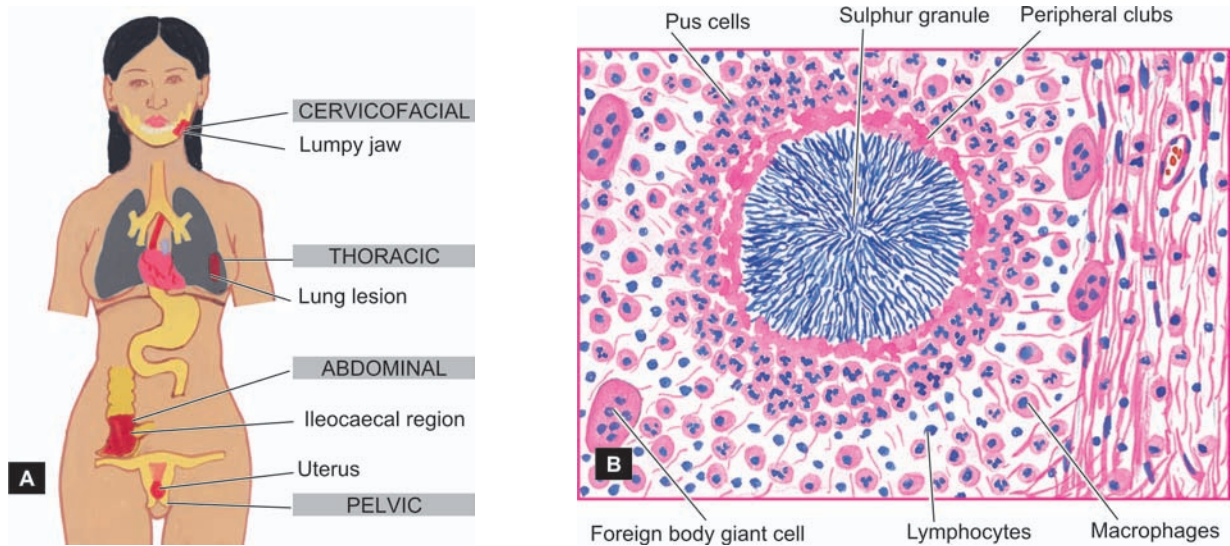


FIGURE 11.13

Morphology of actinomycosis. A, Sites and routes of infection. B, Microscopic appearance of abscess and sulphur granule.

4. Pelvic actinomycosis. Infection in the pelvis occurs as a complication of intrauterine contraceptive devices (IUCD's).

Microscopically, irrespective of the location of actinomycosis, the following features are seen (Fig. 11.13,B) (COLOUR PLATE V: CL 19):

- i) The inflammatory reaction is a granuloma with central suppuration. There is formation of abscesses in the centre of lesions and at the periphery are seen chronic inflammatory cells, giant cells and fibroblasts.
- ii) The centre of each abscess contains the bacterial colony, 'sulphur granule', characterised by radiating filaments (hence previously known as *ray fungus*) with hyaline, eosinophilic, club-like ends representative of secreted immunoglobulins.
- iii) Bacterial stains reveal the organisms as gram-positive filaments, nonacid-fast, which stain positively with Gomori's methenamine silver (GMS) staining.

SARCOIDOSIS (BOECK'S SARCOID)

Sarcoidosis is a systemic disease of unknown etiology. It is worldwide in distribution and affects adults from 20-40 years of age. The disease is characterised by the presence of non-caseating epithelioid cell granulomas ('sarcoid granuloma') in the affected tissues and organs, notably lymph nodes and lungs. Other sites are the skin, spleen, uvea of the eyes, salivary glands, liver and bones of hands and feet. The histologic diagnosis is generally made by exclusion.

ETIOLOGY AND PATHOGENESIS. The cause of sarcoidosis remains unknown. Since the disease is characterised by granulomatous tissue reaction, possibility of cell-mediated immune mechanisms has been suggested. The following observations point towards a possible immune origin of sarcoidosis:

1. Just as in tuberculosis, sarcoidosis is characterised by distinctive granulomatous response against *poorly degradable antigen*, but quite unlike tuberculosis, the antigen in sarcoidosis has eluded workers so far. PCR studies on affected pulmonary tissue have given equivocal result as regards presence of mycobacterial antigen.
2. That there are immunologic abnormalities in sarcoidosis is substantiated by *high levels of CD4+T cells* lavaged from lung lesions. There is also elevation in levels of IL-2 receptors in serum and in lavaged fluid from lungs.
3. There is presence of *activated alveolar macrophages* which elaborate cytokines that initiate the formation of non-caseating granulomas.

PATHOLOGIC CHANGES. The lesions in sarcoidosis are generalised and may affect various organs and tissues at sometime in the course of disease, but brunt of the disease is borne by the lungs and lymph nodes (Fig. 11.14,A):

Microscopically, the following features are present (Fig. 11.14,B).

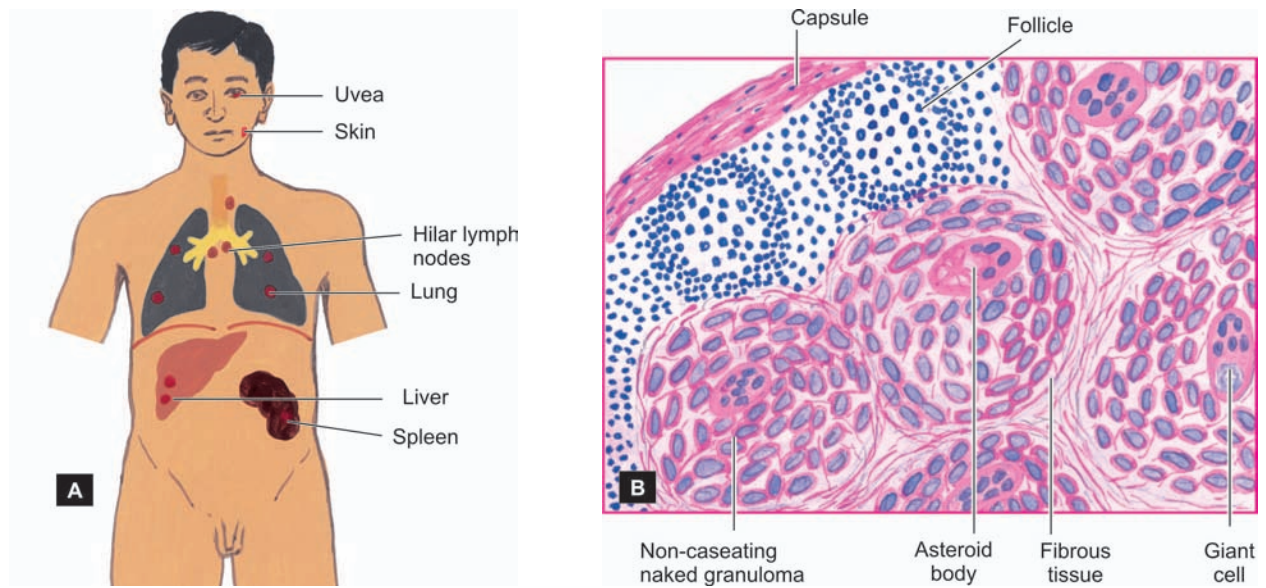


FIGURE 11.14

Morphology of sarcoidosis. A, The lesions are predominantly seen in lymph nodes and throughout lung parenchyma. B, Microscopic appearance of characteristic non-caseating sarcoid granulomas in lymph node.

1. The diagnostic feature in sarcoidosis of any organ or tissue is the *non-caseating sarcoid granuloma*, composed of epithelioid cells, Langhans' and foreign body giant cells and surrounded peripherally by fibroblasts.
2. Typically, granulomas of sarcoidosis are 'naked' i.e. either devoid of peripheral rim of lymphocytes or there is paucity of lymphocytes.
3. In late stage, the granuloma is either *enclosed by hyalinised fibrous tissue* or is replaced by hyalinised fibrous mass.
4. The giant cells in sarcoid granulomas contain certain *cytoplasmic inclusions* like:
 - i) *Asteroid bodies* which are eosinophilic and stellate-shaped structures.

- ii) *Schaumann's bodies or conchoid (conch like) bodies* which are concentric laminations of calcium and of iron salts, complexed with proteins.
- iii) *Birefringent cytoplasmic crystals* which are colourless.

Similar types of inclusions are also observed in chronic berylliosis.

KVIEM'S TEST. It is a useful intradermal diagnostic test. The antigen prepared from involved lymph node or spleen is injected intradermally. In a positive test, nodular lesion appears in 3-6 weeks at the inoculation site which on microscopic examination shows presence of non-caseating granulomas.



Healing of Tissues

Injury to tissue may result in cell death and tissue destruction. *Healing* on the other hand is the body response to injury in an attempt to restore normal structure and function. The process of healing involves 2 distinct processes:

- **Regeneration** when healing takes place by proliferation of parenchymal cells and usually results in complete restoration of the original tissues.

- **Repair** when the healing takes place by proliferation of connective tissue elements resulting in fibrosis and scarring.

At times, both the processes take place simultaneously.

REGENERATION

Some parenchymal cells are short-lived while others have a longer lifespan. In order to maintain proper structure of tissues, these cells are under the constant regulatory control of their cell cycle. These include growth factors such as: epidermal growth factor, fibroblast growth factor, platelet-derived growth factor, endothelial growth factor, transforming growth factor- β . *Cell cycle* (page 23) is defined as the period between two successive cell divisions and is divided into 4 unequal phases (Fig. 12.1):

- **M (mitosis) phase:** Phase of mitosis.
- **G_1 (gap 1) phase:** The daughter cell enters G_1 phase after mitosis.
- **S (synthesis) phase:** During this phase, the synthesis of nuclear DNA takes place.
- **G_2 (gap 2) phase:** After completion of nuclear DNA duplication, the cell enters G_2 phase.
- **G_0 (gap 0) phase:** This is the quiescent or resting phase of the cell after an M phase.

Not all cells of the body divide at the same pace. Some mature cells do not divide at all while others complete a cell cycle every 16-24 hours. The main difference between slowly-dividing and rapidly-dividing cells is the duration of G_1 phase.

Depending upon their capacity to divide, the cells of the body can be divided into 3 groups: labile cells, stable cells, and permanent cells.

1. **Labile cells.** These cells continue to multiply throughout life under normal physiologic conditions. These include: surface epithelial cells of epidermis, alimentary tract, respiratory tract, urinary tract, vagina, cervix, uterine endometrium, haematopoietic cells of bone marrow and cells of lymph nodes and spleen.

2. **Stable cells.** These cells decrease or lose their ability to proliferate after adolescence but retain the capacity to multiply in response to stimuli throughout adult life. These include: parenchymal cells of organs like liver, pancreas, kidneys, adrenal and thyroid; mesenchymal cells like smooth muscle cells, fibroblasts, vascular endothelium, bone and cartilage cells.

3. **Permanent cells.** These cells lose their ability to proliferate around the time of birth. These include: neurons of nervous system, skeletal muscle and cardiac muscle cells.

RELATIONSHIP OF PARENCHYMAL CELLS WITH CELL CYCLE. If the three types of parenchymal cells described above are correlated with the phase of cell cycle (Fig. 12.1), the following inferences can be derived:

1. Labile cells which are continuously dividing cells remain in the cell cycle from one mitosis to the next.
2. Stable cells are in the resting phase (G_0) but can be stimulated to enter the cell cycle.
3. Permanent cells are non-dividing cells which have left the cell cycle and die after injury.

Regeneration of any type of parenchymal cells involves the following 2 processes:

- i) Proliferation of original cells from the margin of injury with migration so as to cover the gap.
- ii) Proliferation of migrated cells with subsequent differentiation and maturation so as to reconstitute the original tissue.

REPAIR

Repair is the replacement of injured tissue by fibrous tissue. Two processes are involved in repair:

1. Granulation tissue formation; and
2. Contraction of wounds.

Repair response takes place by participation of mesenchymal cells (consisting of connective tissue stem

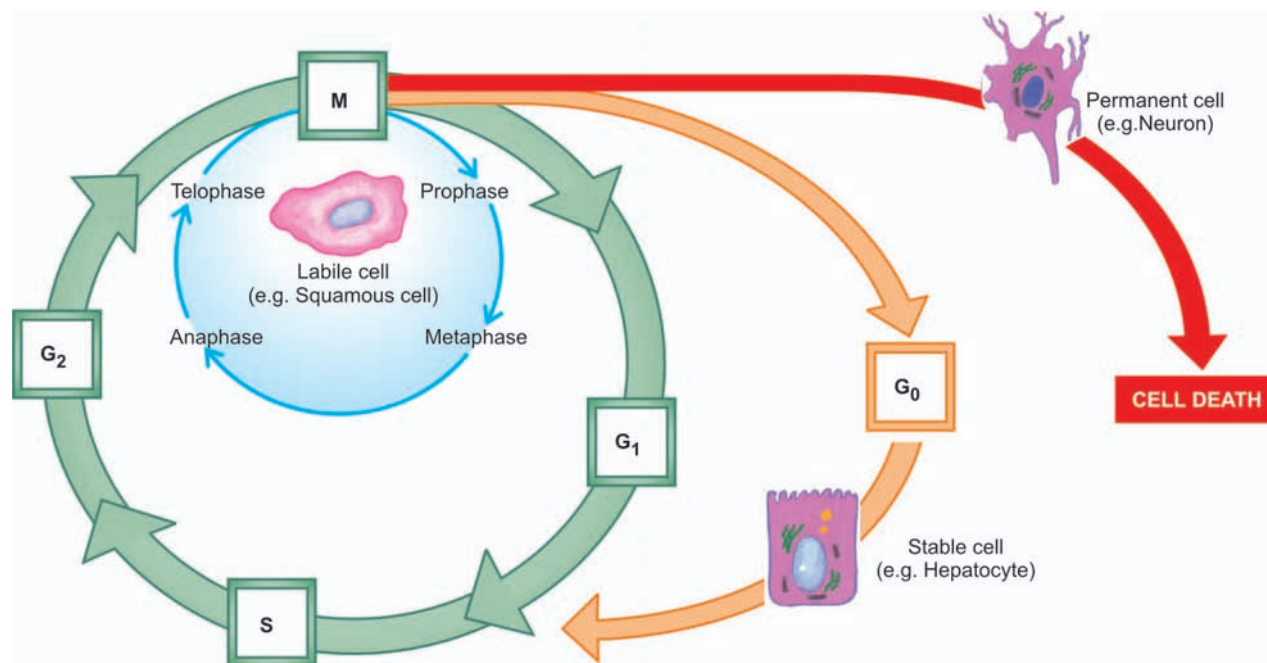


FIGURE 12.1

Parenchymal cells in relation to cell cycle (G₀—Resting phase; G₁, G₂—Gaps; S—Synthesis phase; M—Mitosis phase). The inner circle shown with green line represents cell cycle for labile cells; circle shown with yellow-orange line represents cell cycle for stable cells; and the circle shown with red line represents cell cycle for permanent cells. Compare them with traffic signals—green stands for ‘go’ applies here to dividing labile cells; yellow-orange signal for ‘ready to go’ applies here to stable cells which can be stimulated to enter cell cycle; and red signal for ‘stop’ here means non-dividing permanent cells.

cells, fibrocytes and histiocytes), endothelial cells, macrophages, platelets, and the parenchymal cells of the injured organ.

Granulation Tissue Formation

The term granulation tissue derives its name from slightly granular and pink appearance of the tissue. Each granule corresponds histologically to proliferation of new small blood vessels which are slightly lifted on the surface by thin covering of fibroblasts and young collagen.

The following 3 phases are observed in the formation of granulation tissue (Fig. 12.2):

1. PHASE OF INFLAMMATION. Following trauma, blood clots at the site of injury. There is acute inflammatory response with exudation of plasma, neutrophils and some monocytes within 24 hours.

2. PHASE OF CLEARANCE. Combination of proteolytic enzymes liberated from neutrophils, autolytic enzymes from dead tissues cells, and phagocytic activity of macrophages clear off the necrotic tissue, debris and red blood cells.

3. PHASE OF INGROWTH OF GRANULATION TISSUE.

This phase consists of 2 main processes:

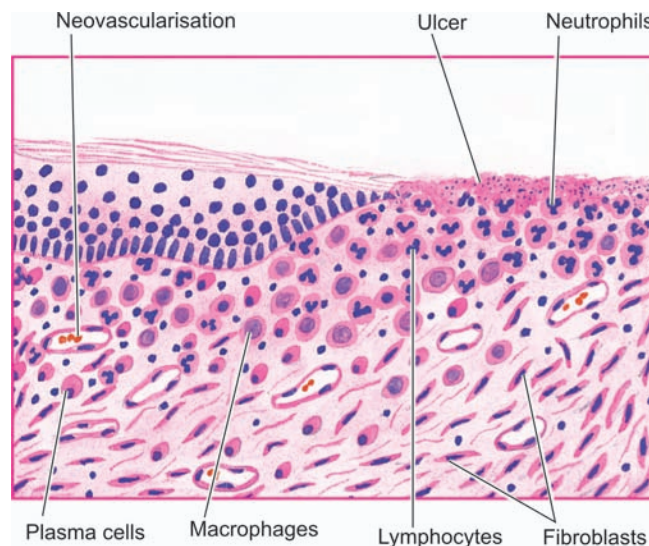


FIGURE 12.2

Active granulation tissue has inflammatory cell infiltrate, newly formed blood vessels and young fibrous tissue in loose matrix.

angiogenesis or neovascularisation, and fibrogenesis (COLOUR PLATE III: CL 12).

i) Angiogenesis (neovascularisation). Formation of new blood vessels at the site of injury takes place by proliferation of endothelial cells from the margins of severed blood vessels. Initially, the proliferated endothelial cells are solid buds but within a few hours develop a lumen and start carrying blood. The newly formed blood vessels are more leaky, accounting for the oedematous appearance of new granulation tissue. Soon, these blood vessels differentiate into muscular arterioles, thin-walled venules and true capillaries.

The process of angiogenesis is stimulated with proteolytic destruction of basement membrane and takes place under the influence of the following factors:

- a) Angiogenesis takes place under the influence of vascular endothelial growth factor (VEGF) elaborated by mesenchymal cells but its receptors are present in endothelial cells only.
- b) Platelet-derived growth factor (PDGF), transforming growth factor- β (TGF- β), basic fibroblast growth factor (bFGF), other cytokines and surface integrins are the factors which are associated with cellular proliferation.

ii) Fibrogenesis. The newly formed blood vessels are present in an amorphous ground substance or matrix. The new fibroblasts originate from fibrocytes as well as by mitotic division of fibroblasts. Some of these fibroblasts have combination of morphologic and functional characteristics of smooth muscle cells (*myofibroblasts*). Collagen fibrils begin to appear by about 6th day. As maturation proceeds, more and more of collagen is formed while the number of active fibroblasts and new blood vessels decreases. This results in formation of inactive looking scar known as *cicatrization*.

Contraction of Wounds

The wound starts contracting after 2-3 days and the process is completed by the 14th day. During this period, the wound is reduced by approximately 80% of its original size. Contracted wound results in rapid healing since lesser surface area of the injured tissue has to be replaced.

In order to explain the mechanism of wound contraction, a number of factors have been proposed. These are as under:

1. *Dehydration* as a result of removal of fluid by drying of wound was first suggested but without being substantiated.
2. *Contraction of collagen* was thought to be responsible for contraction but wound contraction proceeds at a

stage when the collagen content of granulation tissue is very small.

3. Discovery of *myofibroblasts* appearing in active granulation tissue has resolved the controversy surrounding the mechanism of wound contraction. These cells have features intermediate between those of fibroblasts and smooth muscle cells. Their migration into the wound area and their active contraction decreases the size of the defect. The evidences in support of this concept are both morphological as well as functional characteristics of modified fibroblasts or myofibroblasts as under:

- i) Fibrils present in the cytoplasm of these cells resemble those seen in smooth muscle cells.
- ii) These cells contain actin-myosin similar to that found in non-striated muscle cells.
- iii) The nuclei of these cells have infoldings of nuclear membrane like in smooth muscle cells.
- iv) These cells have basement membrane and desmosomes which are not seen in ordinary fibroblasts.
- v) The cytoplasm of these modified cells demonstrates immunofluorescent labelling with anti-smooth muscle antibodies.
- vi) The drug response of granulation tissue is similar to that of smooth muscle.

WOUND HEALING

Healing of skin wounds provides a classical example of combination of regeneration and repair described above. This can be accomplished in one of the following two ways:

- Healing by first intention (*primary union*); and
- healing by second intention (*secondary union*).

Healing by First Intention (Primary Union)

This is defined as healing of a wound which has the following characteristics:

- i) clean and uninfected;
- ii) surgically incised;
- iii) without much loss of cells and tissue; and
- iv) edges of wound are approximated by surgical sutures.

The sequence of events in primary union is illustrated in Fig. 12.3 and described below:

1. Initial haemorrhage. Immediately after injury, the space between the approximated surfaces of incised wound is filled with blood which then clots and seals the wound against dehydration and infection.

2. Acute inflammatory response. This occurs within 24 hours with appearance of polymorphs from the mar-

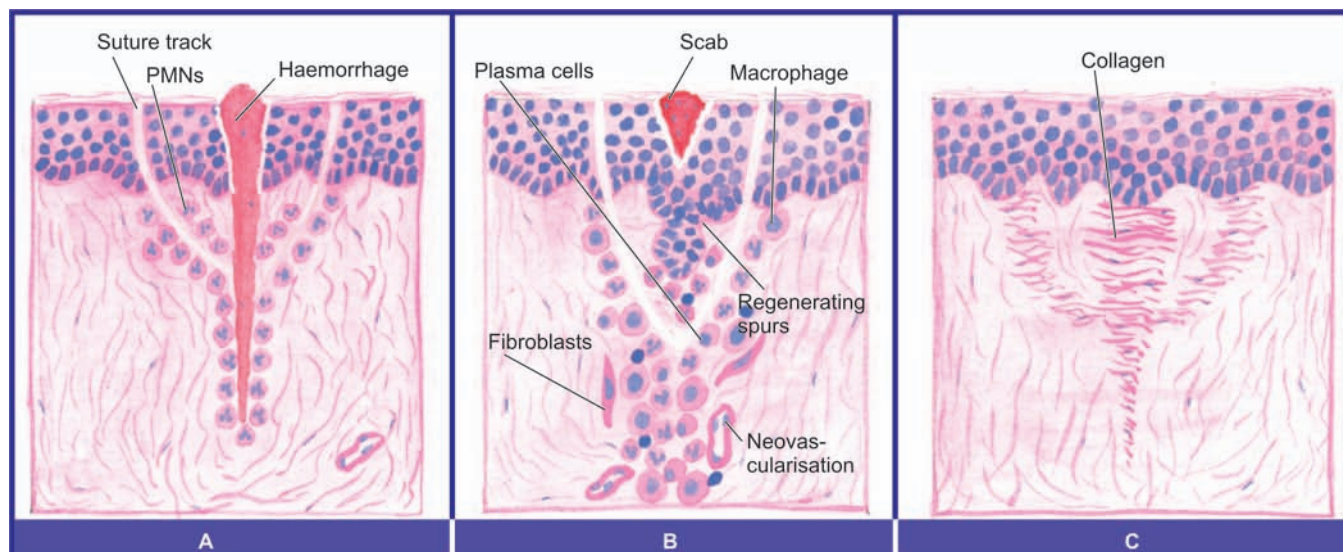


FIGURE 12.3

Primary union of skin wounds. A, The incised wound as well as suture track on either side are filled with blood clot and there is inflammatory response from the margins. B, Spurs of epidermal cells migrate along the incised margin on either side as well as around the suture track. Formation of granulation tissue also begins from below. C, Removal of suture at around 7th day results in scar tissue at the sites of incision and suture track.

gins of incision. By 3rd day, polymorphs are replaced by macrophages.

3. Epithelial changes. The basal cells of epidermis from both the cut margins start proliferating and migrating towards incisional space in the form of epithelial spurs. A well-approximated wound is covered by a layer of epithelium in 48 hours. The migrated epidermal cells separate the underlying viable dermis from the overlying necrotic material and clot, forming *scab* which is cast off. The basal cells from the margins continue to divide. By 5th day, a multilayered new epidermis is formed which is differentiated into superficial and deeper layers.

4. Organisation. By 3rd day, fibroblasts also invade the wound area. By 5th day, new collagen fibrils start forming which dominate till healing is completed. In 4 weeks, the scar tissue with scanty cellular and vascular elements, a few inflammatory cells and epithelialised surface is formed.

5. Suture tracks. Each suture track is a separate wound and incites the same phenomena as in healing of the primary wound i.e. filling the space with haemorrhage, some inflammatory cell reaction, epithelial cell proliferation along the suture track from both margins, fibroblastic proliferation and formation of young collagen. When sutures are removed around 7th day, much of epithelialised suture track is avulsed and the remaining

epithelial tissue in the track is absorbed. However, sometimes the suture track gets infected (*stitch abscess*), or the epithelial cells may persist in the track (*implantation or epidermal cysts*).

Thus, the scar formed in a sutured wound is neat due to close apposition of the margins of wound; the use of adhesive tapes avoids removal of stitches and its complications.

Healing by Second Intention (Secondary Union)

This is defined as healing of a wound having the following characteristics:

- open with a large tissue defect, at times infected;
- having extensive loss of cells and tissues; and
- the wound is not approximated by surgical sutures but is left open.

The basic events in secondary union are similar to primary union but differ in having a larger tissue defect which has to be bridged. Hence healing takes place from the base upwards as well as from the margins inwards. The healing by second intention is slow and results in a large, at times ugly, scar as compared to rapid healing and neat scar of primary union.

The sequence of events in secondary union is illustrated in Fig. 12.4 and described below:

1. Initial haemorrhage. As a result of injury, the wound space is filled with blood and fibrin clot which dries.

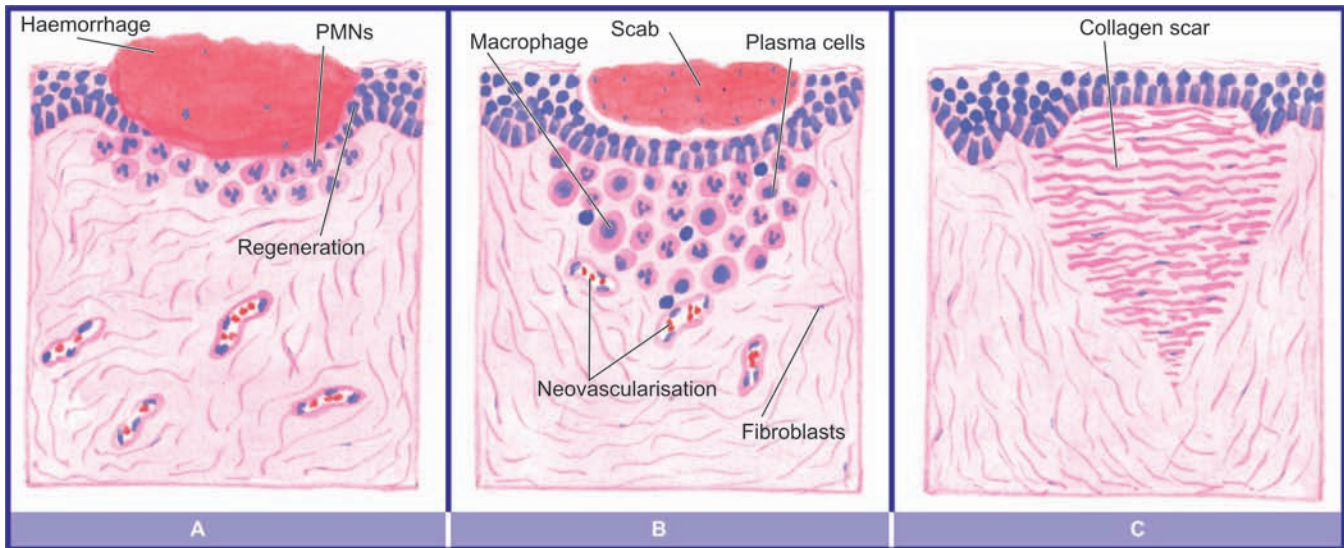


FIGURE 12.4

Secondary union of skin wounds. A, The open wound is filled with blood clot and there is inflammatory response at the junction of viable tissue. B, Epithelial spurs from the margins of wound meet in the middle to cover the gap and separate the underlying viable tissue from necrotic tissue at the surface forming scab. C, After contraction of the wound, a scar smaller than the original wound is left.

2. Inflammatory phase. There is an initial acute inflammatory response followed by appearance of macrophages which clear off the debris as in primary union.

3. Epithelial changes. As in primary healing, the epidermal cells from both the margins of wound proliferate and migrate into the wound in the form of epithelial spurs till they meet in the middle and re-epithelialise the gap completely. However, the proliferating epithelial cells do not cover the surface fully until granulation tissue from base has started filling the wound space. In this way, pre-existing viable connective tissue is separated from necrotic material and clot on the surface, forming *scab* which is cast off. In time, the regenerated epidermis becomes stratified and keratinised.

4. Granulation tissue. The main bulk of secondary healing is by granulations. Granulation tissue is formed by proliferation of fibroblasts and neovascularisation from the adjoining viable elements. The newly-formed granulation tissue is deep red, granular and very fragile. With time, the scar on maturation becomes pale and white due to increase in collagen and decrease in vascularity. The specialised structures of skin like hair follicles and sweat glands are not replaced unless their viable residues remain which may regenerate (COLOUR PLATE III: CL 12).

5. Wound contraction. Contraction of wound is an important feature of secondary healing, not seen in

primary healing. Due to the action of myofibroblasts present in granulation tissue, the wound contracts to one-third to one-fourth of its original size. Wound contraction occurs at a time when active granulation tissue is being formed.

6. Presence of infection. Bacterial contamination of an open wound delays the process of healing due to release of bacterial toxins that provoke necrosis, suppuration and thrombosis. Surgical removal of dead and necrosed tissue, *debridement*, helps in preventing the bacterial infection of open wounds.

Complications of Wound Healing

During the course of healing, following complications may occur:

1. *Infection* of wound due to entry of bacteria delays the healing.
2. *Implantation (epidermal) cyst* formation may occur due to persistence of epithelial cells in the wound after healing.
3. *Pigmentation*. Healed wounds may at times have rust-like colour due to staining with haemosiderin. Some coloured particulate material left in the wound may persist and impart colour to the healed wound.
4. *Deficient scar formation*. This may occur due to inadequate formation of granulation tissue.

5. *Incisional hernia*. A weak scar, especially after a laparotomy, may be the site of bursting open of a wound (wound dehiscence) or an incisional hernia.
6. *Hypertrophied scars and keloid formation*. At times the scar formed is excessive, ugly and painful. Excessive formation of collagen in healing may result in keloid (*claw-like*) formation, seen more commonly in Blacks. Hypertrophied scars differ from keloid in that they are confined to the borders of the initial wound while keloids have tumour-like projection of connective tissue.
7. *Excessive contraction*. An exaggeration of wound contraction may result in formation of contractures or cicatrization e.g. Dupuytren's (palmar) contracture, plantar contracture and Peyronie's disease (contraction of the cavernous tissues of penis)
8. *Neoplasia*. Rarely, scar may be the site for development of carcinoma later e.g. squamous cell carcinoma in Marjolin's ulcer i.e. a scar following burns on the skin.

Extracellular Matrix-Wound Strength

The wound is strengthened by proliferation of fibroblasts and myofibroblasts which get structural support from the extracellular matrix (ECM). In addition to providing structural support, ECM can direct cell migration, attachment, differentiation and organisation.

ECM has five main components: collagen, adhesive glycoproteins, basement membrane, elastic fibres, and proteoglycans.

1. COLLAGEN. The collagens are a family of proteins which provide structural support to the multicellular organism. It is the main component of tissues such as fibrous tissue, bone, cartilage, valves of heart, cornea, basement membrane etc.

Collagen is synthesised and secreted by a complex biochemical mechanism on ribosomes. The collagen synthesis is stimulated by various growth factors and is degraded by collagenase. Regulation of collagen synthesis and degradation take place by various local and systemic factors so that the collagen content of normal organs remains constant. On the other hand, defective regulation of collagen synthesis leads to hypertrophied scar, fibrosis, and organ dysfunction.

Depending upon the biochemical composition, 18 types of collagen have been identified called collagen *type I to XVIII*, many of which are unique for specific tissues. Type I, III and V are *true fibrillar collagen* which form the main portion of the connective tissue during healing of wounds in scars. Other types of collagen are *non-fibrillar* and amorphous material seen as component of the basement membranes.

Morphologically, the smallest units of collagen are *collagen fibrils*, which align together in parallel bundles to form *collagen fibres*, and then *collagen bundles*.

2. ADHESIVE GLYCOPROTEINS. Various adhesive glycoproteins acting as glue for the ECM and the cells consist of fibronectin, tenascin (cytotactin) and thrombospondin.

i) **Fibronectin** (*nectere* = to bind) is the best characterised glycoprotein in ECM and has binding properties to other cells and ECM. It is of two types—plasma and tissue fibronectin.

■ *Plasma fibronectin* is synthesised by the liver cells and is trapped in basement membrane such as in filtration through the renal glomerulus.

■ *Tissue fibronectin* is formed by fibroblasts, endothelial cells and other mesenchymal cells. It is responsible for the primitive matrix such as in the foetus, and in wound healing.

ii) **Tenascin or cytotactin** is the glycoprotein associated with fibroblasts and appears in wound about 48 hours after injury. It disappears from mature scar tissue.

iii) **Thrombospondin** is mainly synthesised by granules of platelets. It functions as adhesive protein for keratinocytes and platelets but is inhibitory to attachment of fibroblasts and endothelial cells.

3. BASEMENT MEMBRANE. Basement membranes are periodic acid-Schiff (PAS)-positive amorphous structures that lie underneath epithelia of different organs and endothelial cells. They consist of collagen type IV and laminin.

4. ELASTIC FIBRES. While the tensile strength in tissue comes from collagen, the ability to recoil is provided by elastic fibres. Elastic fibres consist of 2 components—elastin glycoprotein and elastic microfibril. Elastases degrade the elastic tissue e.g. in inflammation, emphysema etc.

5. PROTEOGLYCANS. These are a group of molecules having 2 components—an essential carbohydrate polymer (called polysaccharide or glycosaminoglycan), and a protein bound to it, and hence the name proteoglycan. Various proteoglycans are distributed in different tissues as under:

- i) *Chondroitin sulphate*—abundant in cartilage, dermis
- ii) *Heparan sulphate*—in basement membranes
- iii) *Dermatan sulphate*—in dermis
- iv) *Keratan sulphate*—in cartilage
- v) *Hyaluronic acid*—in cartilage, dermis.

In wound healing, the deposition of proteoglycans precedes collagen laying.

The strength of wound also depends upon factors like the site of injury, depth of incision and area of

wound. After removal of stitches on around 7th day, the wound strength is approximately 10% which reaches 80% in about 3 months.

Factors Influencing Healing

Two types of factors influence the wound healing: those acting locally, and those acting in general.

A. LOCAL FACTORS. These include the following factors:

1. *Infection* is the most important factor acting locally which delays the process of healing.
2. *Poor blood supply* to wound slows healing e.g. injuries to face heal quickly due to rich blood supply while injury to leg with varicose ulcers having poor blood supply heals slowly.
3. *Foreign bodies* including sutures interfere with healing and cause intense inflammatory reaction and infection.
4. *Movement* delays wound healing.
5. Exposure to *ionising radiation* delays granulation tissue formation.
6. Exposure to *ultraviolet light* facilitates healing.
7. *Type, size and location* of injury determines whether healing takes place by resolution or organisation.

B. SYSTEMIC FACTORS. These are as under:

1. *Age*. Wound healing is rapid in young and somewhat slow in aged and debilitated people due to poor blood supply to the injured area in the latter.
2. *Nutrition*. Deficiency of constituents like protein, vitamin C (scurvy) and zinc delays the wound healing.
3. *Systemic infection* delays wound healing.
4. *Administration of glucocorticoids* has anti-inflammatory effect.
5. *Uncontrolled diabetics* are more prone to develop infections and hence delay in healing.
6. *Haematologic abnormalities* like defect of neutrophil functions (chemotaxis and phagocytosis), and neutropenia and bleeding disorders slow the process of wound healing.

HEALING IN SPECIALISED TISSUES

Healing of skin wound provides an example of general process of healing by regeneration and repair. However, in certain specialised tissues, either regeneration or repair may predominate. Some of these examples are described below.

Fracture Healing

Healing of fracture by callus formation depends upon some clinical considerations whether the fracture is:

- *traumatic* (in previously normal bone), or *pathological* (in previously diseased bone);
- *complete or incomplete* like green-stick fracture; and
- *simple* (closed), *comminuted* (splintering of bone), or *compound* (communicating to skin surface).

However, basic events in healing of any type of fracture are similar and resemble healing of skin wound to some extent.

■ **Primary union of fractures** occurs in a few special situations when the ends of fracture are approximated as is done by application of compression clamps. In these cases, bony union takes place with formation of medullary callus without periosteal callus formation. The patient can be made ambulatory early but there is more extensive bone necrosis and slow healing.

■ **Secondary union** is the more common process of fracture healing. Though it is a continuous process, secondary bone union is described under the following 3 headings:

- i) Procallus formation
- ii) Osseous callus formation
- iii) Remodelling

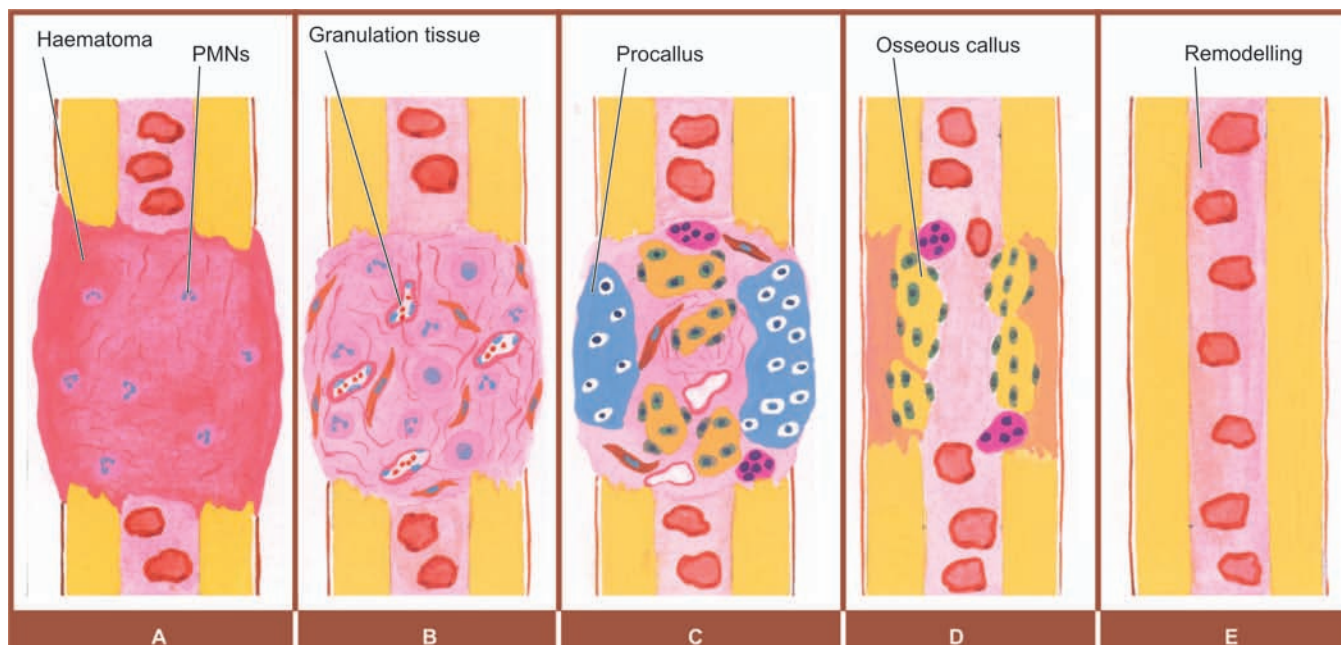
These processes are illustrated in Fig. 12.5 and described below:

I. PROCALLUS FORMATION. Steps involved in the formation of procallus are as follows:

1. **Haematoma** forms due to bleeding from torn blood vessels, filling the area surrounding the fracture (Fig. 12.5,A). Loose meshwork is formed by blood and fibrin clot which acts as framework for subsequent granulation tissue formation.
2. **Local inflammatory response** occurs at the site of injury with exudation of fibrin, polymorphs and macrophages. The macrophages clear away the fibrin, red blood cells, inflammatory exudate and debris. Fragments of necrosed bone are scavenged by macrophages and osteoclasts.

3. **Ingrowth of granulation tissue** begins with neo-vascularisation and proliferation of mesenchymal cells from periosteum and endosteum. A soft tissue callus is thus formed which joins the ends of fractured bone without much strength (Fig. 12.5,B).

4. **Callus composed of woven bone and cartilage** starts within the first few days. The cells of inner layer of the periosteum have osteogenic potential and lay down collagen as well as osteoid matrix in the granulation tissue. The osteoid undergoes calcification and is called *woven bone callus*. A much wider zone over the cortex on either side of fractured ends is covered by the woven bone callus and united to bridge the gap between the

**FIGURE 12.5**

Fracture healing. A, Haematoma formation and local inflammatory response at the fracture site. B, Ingrowth of granulation tissue with formation of soft tissue callus. C, Formation of procallus composed of woven bone and cartilage with its characteristic fusiform appearance and having 3 arbitrary components—external, intermediate and internal callus. D, Formation of osseous callus composed of lamellar bone following clearance of woven bone and cartilage. E, Remodelled bone ends; the external callus cleared away. Intermediate callus converted into lamellar bone and internal callus developing bone marrow cavity.

ends, giving spindle-shaped or fusiform appearance to the union. In poorly immobilised fractures (e.g. fracture ribs), the subperiosteal osteoblasts may form cartilage at the fracture site. At times, callus is composed of woven bone as well as cartilage, temporarily immobilising the bone ends.

This stage is called provisional callus or procallus formation and is arbitrarily divided into *external*, *intermediate* and *internal procallus* (Fig. 12.5,C).

II. OSSEOUS CALLUS FORMATION. The procallus acts as scaffolding on which osseous callus composed of lamellar bone is formed. The woven bone is cleared away by incoming osteoclasts and the calcified cartilage disintegrates. In their place, newly-formed blood vessels and osteoblasts invade, laying down osteoid which is calcified and lamellar bone is formed by developing Haversian system concentrically around the blood vessels (Fig. 12.5,D).

III. REMODELLING. During the formation of lamellar bone, osteoblastic laying and osteoclastic removal are taking place remodelling the united bone ends, which after sometime, is indistinguishable from normal bone. The external callus is cleared away, compact bone (cor-

tex) is formed in place of intermediate callus and the bone marrow cavity develops in internal callus (Fig. 12.5,E).

COMPLICATIONS OF FRACTURE HEALING. These are as under:

1. **Fibrous union** may result instead of osseous union if the immobilisation of fractured bone is not done. Occasionally, a false joint may develop at the fracture site (pseudo-arthritis).
2. **Non-union** may result if some soft tissue is interposed between the fractured ends.
3. **Delayed union** may occur from causes of delayed wound healing in general such as infection, inadequate blood supply, poor nutrition, movement and old age.

Healing of Nervous Tissue

CENTRAL NERVOUS SYSTEM. The nerve cells of brain, spinal cord and ganglia once destroyed are not replaced. Axons of CNS also do not show any significant regeneration. The damaged neuroglial cells, however, may show proliferation of astrocytes called gliosis.

PERIPHERAL NERVOUS SYSTEM. The peripheral nerves, unlike brain, have regenerative capacity. The

pathologic reactions of the PNS in response to injury may be in the form of one of the types of degenerations causing *peripheral neuropathy* or formation of a *traumatic neuroma*. There are 3 main types of degenerative processes in the PNS—Wallerian degeneration, axonal degeneration and segmental demyelination.

Wallerian degeneration. Wallerian degeneration occurs after transection of the axon which may be as a result of knife wounds, compression, traction and ischaemia. Following transection, initially there is accumulation of organelles in the proximal and distal ends of the transection sites. Subsequently, the axon and myelin sheath distal to the transection site undergo disintegration upto the next node of Ranvier, followed by phagocytosis (Fig. 12.6,B). The process of regeneration occurs by sprouting of axons and proliferation of Schwann cells from the proximal end.

Axonal degeneration. In axonal degeneration, degeneration of the axon begins at the peripheral terminal and proceeds backward towards the nerve cell body (Fig. 12.6,C). The cell body often undergoes chromatolysis. There is Schwann cell proliferation in the region of axonal degeneration. The loss of axonal integrity occurs,

probably as a result of some primary metabolic disturbance within the axon itself. Changes similar to those seen in Wallerian degeneration are present but regenerative reaction is limited or absent.

Segmental demyelination. It is similar to demyelination within the brain. Segmental demyelination is demyelination of the segment between two consecutive nodes of Ranvier, leaving a denuded axon segment. The axon, however, remains intact. Schwann cell proliferation generally accompanies demyelination (Fig. 12.6,D). This results in remyelination of the affected axon. Repeated episodes of demyelination and remyelination are associated with concentric proliferation of Schwann cells around axons producing '*onion bulbs*' found in hypertrophic neuropathy.

Traumatic neuroma. Normally, the injured axon of a peripheral nerve regenerates at the rate of approximately 1 mm per day. However, if the process of regeneration is hampered due to an interposed haematoma or fibrous scar, the axonal sprouts together with Schwann cells and fibroblasts form a peripheral mass called as traumatic or stump neuroma.

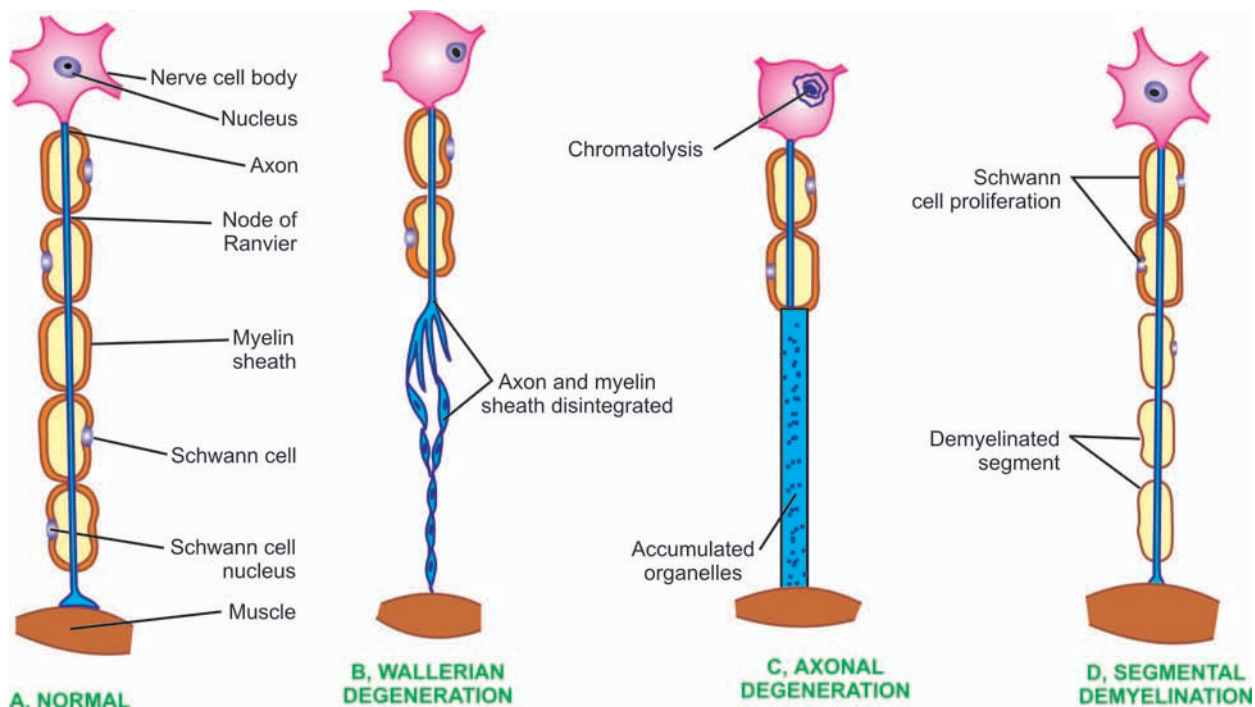


FIGURE 12.6

Pathologic reaction of peripheral nerve to injury.

Healing of Muscle

All three types of muscle fibres have limited capacity to regenerate.

SKELETAL MUSCLE. The regeneration of striated muscle is similar to peripheral nerves. On injury, the cut ends of muscle fibres retract but are held together by stromal connective tissue. The injured site is filled with fibrinous material, polymorphs and macrophages. After clearance of damaged fibres by macrophages, one of the following two types of regeneration of muscle fibres can occur:

- If the muscle sheath is intact, sarcolemmal tubes containing histiocytes appear along the endomysial tube which, in about 3 months time, restores properly oriented muscle fibres e.g. in Zenker's degeneration of muscle in typhoid fever.

- If the muscle sheath is damaged, it forms a disorganised multinucleate mass and scar composed of fibrovascular tissue e.g. in Volkmann's ischaemic contracture.

SMOOTH MUSCLE. Non-striated muscle has limited regenerative capacity e.g. appearance of smooth muscle in the arterioles in granulation tissue. However, in large destructive lesions, the smooth muscle is replaced by permanent scar tissue.

CARDIAC MUSCLE. Destruction of heart muscle is replaced by fibrous tissue. However, in situations where

the endomysium of individual cardiac fibre is intact (e.g. in diphtheria and coxsackie virus infections), regeneration of cardiac fibres may occur in young patients.

Healing of Mucosal Surfaces

The cells of mucosal surfaces have very good regeneration and are normally being lost and replaced continuously e.g. mucosa of alimentary tract, respiratory tract, urinary tract, uterine endometrium etc. This occurs by proliferation from margins, migration, multilayering and differentiation of epithelial cells in the same way as in the epidermal cells in healing of skin wounds.

Healing of Solid Epithelial Organs

Following gross tissue damage to organs like kidney, liver and thyroid, the replacement is by fibrous scar e.g. in chronic pyelonephritis and cirrhosis of liver. However, in parenchymal cell damage with intact basement membrane or intact supporting stromal tissue, regeneration may occur. For example:

- *In tubular necrosis* of kidney with intact basement membrane, proliferation and slow migration of tubular epithelial cells may occur to form renal tubules.

- *In viral hepatitis* if part of the liver lobule is damaged with intact stromal network, proliferation of hepatocytes may result in restoration of liver lobule.



Immunity and immunopathology are proverbial two edges of 'double-edged sword'.

Before discussing immunopathology which is the study of derangements in the immune system, it is important to know the structure and function of the normal immune system (immunophysiology) and to get familiarised with a few terms and definitions commonly used in any description of immunology.

■ An **antigen (Ag)** is defined as a substance, usually protein in nature, which when introduced into the tissues stimulates antibody production.

■ **Hapten** is a non-protein substance which has no antigenic properties, but on combining with a protein can form a new antigen capable of forming antibodies.

■ An **antibody (Ab)** is a protein substance produced as a result of antigenic stimulation. Circulating antibodies are immunoglobulins (Igs) of which there are 5 classes: IgG, IgA, IgM, IgE and IgD.

■ Alternatively, an antigen may induce **specifically sensitised cells** having the capacity to recognise, react and neutralise the injurious agent or organisms.

■ The antigen may combine with antibody to form **antigen-antibody complex**. The reaction of Ag with Ab *in vitro* may be *primary* or *secondary phenomena*; the secondary reaction induces a number of processes. *In vivo*, the Ag-Ab reaction may cause tissue damage.

COMPONENTS OF IMMUNE SYSTEM

Broadly speaking, immunity or body defense mechanism is divided into 2 types, each with humoral and cellular components:

I. Nonspecific or innate immunity is considered as the first line of defense without antigenic specificity. Its major components are:

- a) *Humoral*: comprised by complement.
- b) *Cellular*: consists of neutrophils, macrophages, and natural killer (NK) cells.

II. Specific or adaptive immunity is characterised by antigenic specificity. Its main components are:

- a. *Humoral*: consisting of antibodies formed by B cells.
- b. *Cellular*: mediated by T cells.

The various components of both types of immunity are interdependent and interlinked for their functions.

The components of innate immunity are the complement, neutrophils and macrophages are described in Chapter 10. The other components of the immune system are considered below:

Lymphocytes

Morphologically, lymphocytes appear as a homogeneous group but functionally two major lymphocyte populations are identified—T and B lymphocytes. B cells differentiate into plasma cells which form specific antibodies. T cells proliferate on coming in contact with appropriate antigen after which the non-specific accessory cells (PMNs and macrophages) play a critical role in completion of immunopathologic reaction.

B and T cells cannot be distinguished by light or electron microscopy but can be differentiated by immunologic methods (Table 13.1). B cells express specific antibodies as immunoglobulins. T cells, on the other hand, have surface molecules expressed at different stages of their development which are characterised by monoclonal antibodies according to the *cluster of differentiation* (CD).

Hybridoma Monoclonal Antibodies

Hybridoma technique is the *in vitro* method of obtaining monoclonal antibodies against any antigen by mixing (hybridising) B cells of required clone of antibodies with myeloma cells as source for continued secretion of immunoglobulin to immortalise the cell lineage. Currently, hybridoma monoclonal antibodies are produced commercially against all possible antigens such as DNA, RNA, intermediate filaments, molecular cellular proteins etc. The use of hybridoma monoclonal antibodies has helped in recognising subpopulations of T and B cells. This has been found particularly helpful in identification of benign and malignant lesions of B cell series. Antibodies labelled specifically to kappa (κ) and lambda (λ) light chains, both of which are normally present on B cells, can be used to determine whether the lesion is:

■ *polyclonal*, if arising from stimulation of several clones containing κ as well as λ light chains e.g. in non-neoplastic or reactive proliferations; or

TABLE 13.1: Differences between T and B Lymphocytes.

FEATURE	T CELLS	B CELLS
1. <i>Origin</i>	Bone marrow → Thymus	Bone marrow → Bursa (in fowl); mucosa-associated lymphoid tissue (MALT)
2. <i>Life span</i>	Small T cells: months to years T cell blasts: several days	Small B cells: less than 1 month B cell blasts : several days
3. <i>Location</i>		
(i) <i>Lymph nodes</i>	Perifollicular (paracortical)	Germinal centres, medullary cords
(ii) <i>Spleen</i>	Periarteriolar	Germinal centres, red pulp
(iii) <i>Peyer's patches</i>	Perifollicular	Central follicles
4. <i>Presence in</i>		
(i) <i>Blood</i>	80%	20%
(ii) <i>Bone marrow</i>	Rarely present	Numerous
(iii) <i>Lymph nodes</i>	85%	15%
(iv) <i>Spleen</i>	65%	35%
(v) <i>Thymus</i>	90%	10%
5. <i>Surface markers</i>		
(i) <i>Ag receptors</i>	Present	Absent
(ii) <i>Surface Ig</i>	Absent	Present
(iii) <i>Fc receptor</i>	Absent	Present
(iv) <i>Complement receptor</i>	Absent	Present
(v) <i>Rosettes</i>	E-rosettes (sheep erythrocytes)	EAC-rosettes (mouse erythrocytes)
(vi) <i>CD markers</i>	TH CD4, 3, 7, 2 TS CD8, 3, 7, 2	CD19, 21, 23
6. <i>Functions</i>	(i) CMI via cytotoxic T cells positive for CD3 and CD4 (ii) Delayed hypersensitivity via CD4+ T cells (iii) Immunoregulation of other T cells, B cells and stem cells via T helper (CD4+) or T suppressor (CD8+) cells	(i) Role in humoral immunity by synthesis of specific antibodies (Igs) (ii) Precursors of plasma cells

■ *monoclonal*, if arising from stimulation of only one clone and thus one light chain type only e.g. in malignant tumours.

Effector Lymphocyte Subpopulations

Different subpopulations of sensitised lymphocytes (T_{DTH} , CD4+ T cells, or helper T cells; T_{CTL} , CD8+ T cells, or cytotoxic lymphocytes) and unsensitised cells (NK or natural killer cells) can mediate the specific immune responses (Table 13.2).

Macrophage

The role of macrophages in inflammation consisting of circulating monocytes and tissue macrophages has been described in Chapter 10. The macrophage subpopulations like the dendritic cells found in the lymphoid tissue and Langerhans' cells seen in the

epidermis, are characterised by the presence of dendritic cytoplasmic processes and are active in the immune system.

The functions of macrophage subpopulations in the immune system are as follows:

- They *recognise the antigen* for presenting to immuno-competent T or B cells.
- The *phagocytic function* of macrophage is performed due to location of surface receptor for Fc fragment of IgG and C_3 complement.
- Macrophages produce *interleukin-1* which has a role in differentiation stage of T and B lymphocytes.
- Macrophages are capable of *lysing tumour cells* by secretion of toxic metabolites.
- They act as powerful effector cells in some forms of *cell-mediated immunity* e.g. in delayed hypersensitivity reactions.

TABLE 13.2: Effector Lymphocyte Subpopulations.

SUBPOPULATION	FUNCTIONS
1. T_{DTH} (CD4+, helper T) cells	60% peripheral T cells Release lymphokines Activate macrophages Mediate delayed hypersensitivity reactions
2. T_{CTL} (CD8+, cytotoxic suppressor T) cells	30% peripheral T cells Specific cell-mediated cytotoxicity
3. NK (CD11, 45, 46, 57) cells	React without previous sensitisation React and lyse the target cells e.g. tumour cells, virus-infected cells and some normal cells
4. K (Fc Ig receptor) cells	Antibody-dependent cell-mediated cytotoxicity e.g. autoimmune thyroiditis
5. TIL (activated NK) cells	Lysis of cells more effective than NK cells

CD= Cluster of differentiation; CTL= cytotoxic T lymphocytes; NK= natural killer; K= killer; TIL= tumour infiltrating lymphocytes; Fc Ig= crystallisable fragment of immunoglobulin.

vi) They act as *mediators of inflammation* in release of prostaglandins, activation of coagulation factors and in secretion of acute phase reactant proteins by the liver.

HUMAN LEUCOCYTE ANTIGEN (HLA) SYSTEM

Though not a component of immune system, HLA system is described here as it is considered important in the regulation of the immune system. HLA stands for Human Leucocyte Antigens because these antigens were first discovered on leucocytes. The major importance of human histocompatibility antigens of HLA system lies in matching donor and recipient for organ transplant.

Out of the various genes for histocompatibility antigens, most of the transplantation antigens are located on a portion of *chromosome 6* of all nucleated cells of the body and platelets. This is called *major histocompatibility complex (MHC) or HLA complex*. These genes occupy four regions or loci—A, B, C and D, on the short (p) arm of chromosome 6 and exhibit marked variation in allelic genes at each locus. Therefore, the product of HLA antigens is highly polymorphic. The letter w in some of the genes (e.g. D_{w3} , C_{w4} , B_{w15} etc) refers to the numbers allocated to them at international workshops.

Depending upon the characteristics of MHC, they have been divided into 3 classes (Fig. 13.1):

■ **Class I MHC antigens** have loci as HLA-A, HLA-B and HLA-C. CD8 lymphocytes carry receptors for class I MHC and are used to identify the cells having them.

■ **Class II MHC antigens** have single locus as HLA-D. These antigens have further 3 loci, DR, DQ and DP. Class II MHC is identified by B cells and CD4 or T helper cells.

■ **Class III MHC antigens** are some components of the complement system (C2 and C4) coded on HLA

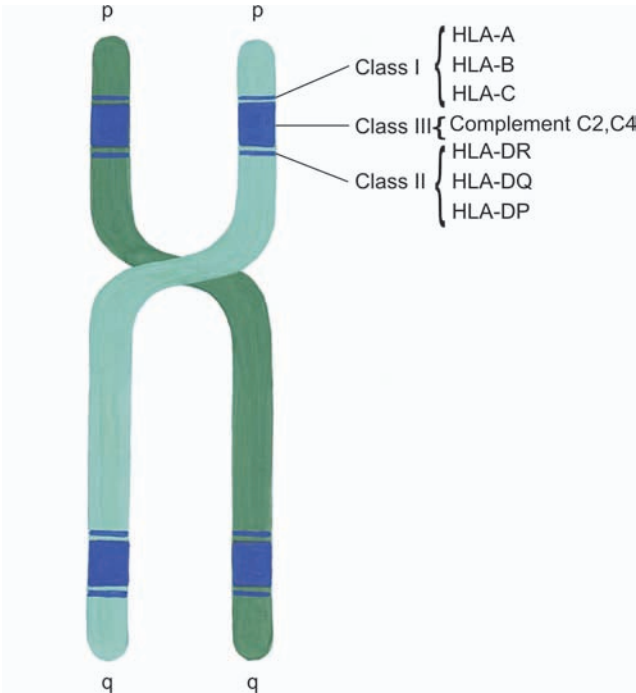


FIGURE 13.1
HLA system and loci on chromosome 6.

complex but are not associated with HLA expression and are not used in antigen identification.

In view of high polymorphism of class I and class II genes, they have a number of alleles on loci numbered serially like HLA-A 1, HLA-A 2, HLA-A 3 etc.

ROLE OF HLA COMPLEX. The HLA complex is significant in a number of ways:

1. Organ transplantation. Historically, the major importance of HLA system is in matching donor and recipient for tissue transplantation. The recipient's immune system can recognise the histocompatibility antigens on the donor organ and accordingly accept it or reject it. Both humoral as well as cell-mediated immune responses are involved in case of genetically non-identical transplants.

2. Regulation of the immune system. Class I and II histocompatibility antigens play a role in regulating both cellular and humoral immunity:

■ *Class I* Ags regulate the function of cytotoxic T cells (CD8+ subpopulation) e.g. in virus infections.

■ *Class II* Ags regulate the function of helper T cells (CD4+ subpopulation).

3. Association of diseases with HLA. An increasing number of diseases has been found to have association with some specific histocompatibility antigens. These disorders include the following:

- i) *Inflammatory* such as ankylosing spondylitis.
- ii) *Autoimmune disorders* such as rheumatoid arthritis, insulin-dependent diabetes mellitus.
- iii) *Inherited disorders of metabolism* like idiopathic haemochromatosis.

The exact mechanism of such associations between the disease and HLA type is not clearly understood.

DISEASES OF IMMUNITY

The word *immunity* is synonymous with *resistance* meaning protection from particular diseases or injuries, whereas the term *hypersensitivity* is interchangeable with *allergy* meaning a state of exaggerated or altered immune response to a given agent. The diseases of the immune system are broadly classified into the following 4 groups:

I. Immunodeficiency disorders characterised by deficient cellular and/or humoral immune functions. This group is comprised by a list of *primary and secondary immunodeficiency diseases* including the dreaded *acquired immunodeficiency syndrome (AIDS)*.

II. Hypersensitivity reactions characterised by hyperfunction of the immune system and cover the various mechanisms of *immunologic tissue injury*.

III. Autoimmune diseases occur when the immune system fails to recognise 'self' from 'non-self'. A growing number of *autoimmune and collagen diseases* are included in this group.

IV. Possible immune disorders in which the immunologic mechanisms are suspected in their etiopathogenesis. Classical example of this group is *amyloidosis* discussed in Chapter 7.

In addition to the above groups of immunologic disorders, an account of *transplantation rejection* is appended at the end of discussion below.

IMMUNODEFICIENCY DISEASES

Failure or deficiency of immune system, which normally plays a protective role against infections, manifests by occurrence of repeated infections in an individual having immunodeficiency diseases.

Traditionally, immunodeficiency diseases are classified into 2 types:

A. *Primary immunodeficiencies* are usually the result of genetic or developmental abnormality of the immune system.

B. *Secondary immunodeficiencies* arise from acquired suppression of the immune system.

Since the first description of primary immunodeficiency by Bruton in 1952, an increasing number of primary and secondary immunodeficiency syndromes are being added to the list, the latest addition being the acquired immunodeficiency syndrome (AIDS) in 1981.

A list of most immunodeficiency diseases with the possible defect in the immune system is given in Table 13.3, while an account of AIDS is given below.

ACQUIRED IMMUNODEFICIENCY SYNDROME (AIDS)

Since the initial recognition of AIDS in the United States in 1981, tremendous advances have taken place in the understanding of this dreaded disease in the last decade as regards its epidemiology, etiology, immunology, pathogenesis, clinical features and morphologic changes in various tissues and organs of the body. But efforts at finding its definite treatment and a vaccine have not yielded success so far, and thus the prognosis remains grim. Hence the global attention is presently focussed on preventive measures.

EPIDEMIOLOGY. Although AIDS was first described in the US, the disease has now attained pandemic proportions involving all continents. Presently, developing countries comprise majority of cases and Africa alone constitutes 50% of all positive cases globally. According to a rough estimate, 1 in every 100 sexually active adults

TABLE 13.3: Immunodeficiency Diseases.

DISEASE	DEFECT
A. PRIMARY IMMUNODEFICIENCY DISEASES	
1. Severe combined immunodeficiency diseases (Combined deficiency of T cells, B cells and Igs):	
(i) Reticular dysgenesis	Failure to develop primitive marrow reticular cells
(ii) Thymic aplasia	No lymphoid stem cells
(iii) Agammaglobulinaemia (Swiss type)	No lymphoid stem cells
(iv) Wiscott-Aldrich syndrome	Cell membrane defect of haematopoietic stem cells; associated features are thrombocytopenia and eczema
(v) Ataxia telangiectasia	Defective T cell maturation
2. T cell defect:	
DiGeorge's syndrome (thymic hypoplasia)	Epithelial component of thymus fails to develop
3. B cell defects (antibody deficiency diseases):	
(i) Bruton's X-linked agammaglobulinaemia	Defective differentiation from pre-B to B cells
(ii) Autosomal recessive agammaglobulinaemia	Defective differentiation from pre-B to B cells
(iii) IgA deficiency	Defective maturation of IgA synthesising B cells
(iv) Selective deficiency of other Ig types	Defective differentiation from B cells to specific Ig-synthesising plasma cells
(v) Immune deficiency with thymoma	Defective pre-B cell maturation
4. Common variable immunodeficiencies (characterised by decreased Igs and serum antibodies and variable CMI):	
(i) With predominant B cell defect	Defective differentiation of pre-B to mature B cells
(ii) With predominant T cell defect	
(a) Deficient T helper cells	Defective differentiation of thymocytes to T helper cells
(b) Presence of activated T suppressor cells	T cell disorder of unknown origin
(iii) With autoantibodies to B and T cells	Unknown differentiation defect
B. SECONDARY IMMUNODEFICIENCY DISEASES	
1. Infections	AIDS (HIV virus); other viral, bacterial and protozoal infections
2. Cancer	Chemotherapy by antimetabolites; irradiation
3. Lymphoid neoplasms (lymphomas, lymphoid leukaemias)	Deficient T and B cell functions
4. Malnutrition	Protein deficiency
5. Sarcoidosis	Impaired T cell function
6. Autoimmune diseases	Administration of high dose of steroids toxic to lymphocytes
7. Transplant cases	Immunosuppressive therapy

worldwide is infected with HIV. Half of all serologically positive cases are in women while children comprise 5% of all cases. According to the WHO data, the last decade has shown an alarming rise in incidence of AIDS cases in South-East Asia including Thailand, Indonesia and Indian sub-continent. However, giving exact figures of cases is pointless since the numbers are increasing by millions and all such data will be outdated with every passing year. In India, epicentre of the epidemic lies in the states of Maharashtra and Tamil Nadu which together comprise about 50% of all HIV positive cases (mostly contracted sexually), while North-East state of Manipur accounts for 8% of all cases (mostly among intravenous drug abusers).

ETIOLOGIC AGENT. AIDS is caused by a retrovirus called human immunodeficiency virus (HIV) which is a type of human T cell leukaemia-lymphoma virus (HTLV).

HIV resembles other HTLVs in shape and size and both have *tropism for CD4 molecules* present on subpopulation of T cells which are the particular targets of attack by HIV. However, *HIV differs from HTLV in being cytolytic for T cells causing immunodeficiency (cytopathic virus) while HTLV may transform the target cells into T cell leukaemia (transforming virus)* (Chapter 21). Two forms of HIV have been described, HIV1 being the etiologic agent for AIDS in the US and Central Africa, while HIV2 causes a similar disease in West Africa and parts of India.

HIV-I virion or virus particle is spherical in shape and 100-140 nm in size (Fig. 13.2):

■ It contains a *core* having *core proteins*, chiefly p24 and p18, two strands of genomic RNA and the enzyme, reverse transcriptase.

■ The core is covered by a *double layer of lipid membrane* derived from the outer membrane of the infected host

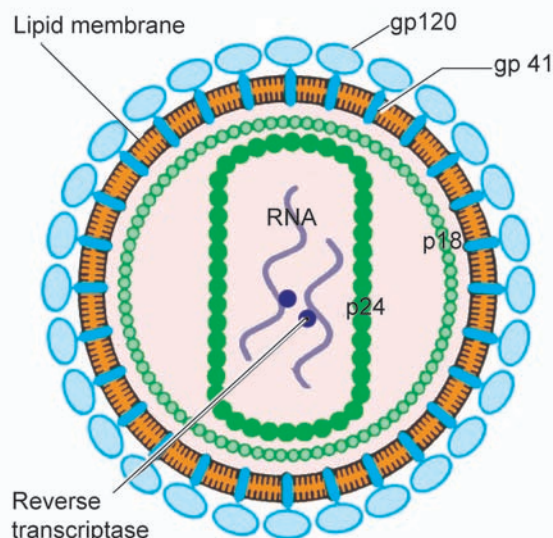


FIGURE 13.2

Schematic representation of HIV virion or virus particle. The particle has core containing proteins, p24 and p18, two strands of viral RNA, and enzyme reverse transcriptase. Bilayer lipid membrane is studded with 2 viral glycoproteins, gp120 and gp41, in the positions shown.

cell during budding process of virus. The membrane is studded with 2 *viral glycoproteins*, gp120 and gp41, in the positions shown.

Besides various other genes, **three important genes** which code for the respective components of virion are: *gag* for core proteins, *pol* for reverse transcriptase, and *env* for the envelope proteins. These genes and viral components act as markers for the laboratory diagnosis of HIV infection. Besides, there is *tat* gene (transactivator) for viral functions such as amplification of viral genes, viral budding and replication.

ROUTES OF TRANSMISSION. Transmission of HIV infection occurs by one of following three routes:

1. Sexual contact. Sexual contact is the main mode of spread and constitutes 75% of all cases of HIV transmission. Most cases of AIDS in the industrialised world like in the US occur in *homosexual or bisexual males* while *heterosexual promiscuity* seems to be the dominant mode of HIV infection in Africa and Asia. Other sexually transmitted diseases (STDs) may act as cofactors for spread of HIV, in particular gonorrhoea and chlamydia. Transmission from male-to-male and male-to-female is more potent route than that from female-to-male.

2. Parenteral transmission. This mode of transmission is the next largest group (25%) and occurs in 3 groups of high risk populations:

- i) *Intravenous drug abusers* by sharing needles, syringes etc comprise a large group in the US.
- ii) *Haemophiliacs* who have received large amounts of factor VIII concentrates from pooled blood components from multiple donors.
- iii) *Recipients of blood and blood products* who have received multiple transfusions of whole blood or components like platelets and plasma.

3. Perinatal transmission. HIV infection occurs from infected mother to the newborn during pregnancy *transplacentally*, or in immediate post-partum period *through contamination* with maternal blood, infected amniotic fluid or breast milk.

In about 6% of cases, the risk factor cannot be determined.

Besides blood, *HIV has been isolated from a number of body fluids and tissues* such as: semen, vaginal secretions, cervical secretions, breast milk, CSF, synovial, pleural, peritoneal, pericardial and amniotic fluid.

In order to allay the fear in the public regarding spread of HIV infection, *AIDS cannot be transmitted* by casual non-sexual contact like shaking hands, hugging, sharing household facilities like beds, toilets, utensils etc.

HIV contaminated waste products can be *sterilised and disinfected* by most of the chemical germicides used in laboratories at a much lower concentration. These are: sodium hypochlorite (liquid chlorine bleach), formaldehyde (5%), ethanol (70%), glutaraldehyde (2%), β -propiolactone. HIV is also heat-sensitive and can be inactivated at 56°C for 30 min.

PATHOGENESIS. The pathogenesis of HIV infection is largely related to the *depletion of CD4+ T cells (helper T cells) resulting in profound immunosuppression*.

The sequence of events shown in Fig. 13.3 is outlined below:

1. Selective tropism and internalisation. HIV on entering the body via any route described above has *selective tropism for CD4+ cells* and certain population of monocytes and macrophages. In this way, envelope glycoprotein gp120 of the virion binds to CD molecule and the virus particle is internalised into CD4+ cell.

2. Uncoating and proviral DNA integration. The virus is then uncoated and its genomic RNA is transcribed to proviral DNA by enzyme reverse transcriptase. The proviral DNA so formed may initially remain *unintegrated* in the affected cell and later gets *integrated* into the T cell genome.

3. Budding and syncytia formation. On activation, the infected CD4+ T cells bear budding viral particles due

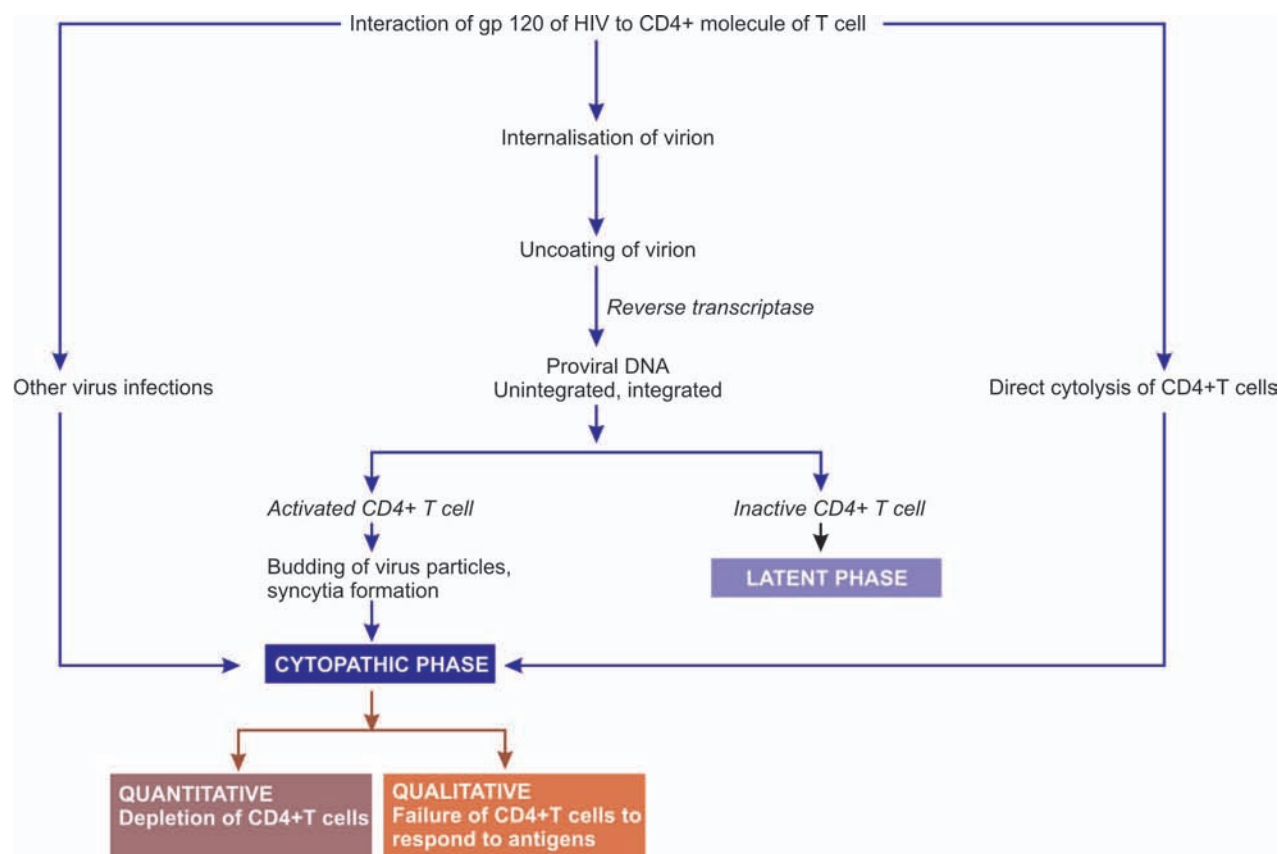


FIGURE 13.3

Sequence of events in the pathogenesis of HIV infection.

to multiplication which further attract more number of CD4+ T cells resulting in syncytia formation.

4. Cytopathic effects. In an inactive T cell, the infection may remain in *latent phase* for a long time, accounting for the long incubation period. An antigenically-activated and infected T cell develops into *cytopathic phase*. How cytopathic effects on T cells are produced is not quite clear. However, there is evidence to suggest that these effects occur due to interaction of CD4 molecule of T cell with gp120 of HIV viral envelope. The cytopathic effects on T cells are two fold: *firstly*, there is quantitative depletion of CD4+ T cells due to direct cytolysis; and *secondly*, there is qualitative defect in the form of inability of these T cells to respond to antigens.

The CD4 molecule-gp120 interaction also leads to *infection with other viruses* like CMV, hepatitis, herpes simplex etc.

5. Effects on monocytes and macrophages. Besides the cytopathic effects on CD4+ T cells, CD4 molecule-bearing subpopulations of monocytes and macrophages

(e.g., dendritic cells, microglial cells) are also attacked by HIV but without causing cytopathic effects on these cells. These HIV-infected cells, thus, may act as a reservoir of HIV infection as well as may be the source of infection to other organs like the nervous system.

6. HIV infection of nervous system. Out of non-lymphoid organ involvement, HIV infection of nervous system is the most serious and can occur as a result of the following:

- i) Infection carried to the microglia of the nervous system by *HIV infected CD4+ monocyte-macrophage subpopulation or endothelial cells*.
- ii) *Direct infection* of astrocytes and oligodendrocytes.
- iii) Neurons are not invaded by HIV but are affected due to gp120 and by release of *cytokines* by macrophages.

75-90% of AIDS patients may demonstrate some form of neurological involvement at autopsy. Various syndromes which may appear as neurological manifestations and sometimes presenting features of AIDS include the following:

- acute aseptic meningitis;
- subacute encephalitis;
- vacuolar myelopathy; and
- peripheral neuropathy.

7. B cell dysfunctions. Besides affecting CD4⁺ cells, gp 120 of HIV envelope also produces derangements of B cell functions resulting in decreased immunoglobulin production, hypergammaglobulinaemia, polyclonal activation, circulating immune complexes.

A summary of major abnormalities in the immune system in AIDS is given in Table 13.4.

NATURAL HISTORY. As per the WHO, AIDS is defined as the existence of at least two major signs associated with at least one minor sign, in the absence of known secondary causes of immunosuppression.

■ *Major signs* include:

- i) weight loss of > 10% of body weight;
- ii) chronic diarrhoea of > 1 month's duration; and
- iii) prolonged fever (intermittent or continuous) for > 1 month.

■ *Minor signs* are:

- i) recurrent oropharyngeal candidiasis;
- ii) persistent generalised lymphadenopathy;
- iii) persistent cough for > one month;
- iv) generalised pruritic dermatitis;
- v) recurrent herpes zoster; and
- vi) progressive disseminated herpes simplex infection.

The following 3 phases are recognised (Table 13.5):

1. Acute HIV syndrome (3-12 weeks). Entry of HIV into the body of an immunocompetent host is heralded by the following sequence of events:

- i) High levels of plasma *viraemia* due to replication of the virus.
- ii) Virus-specific immune response as seen by *seroconversion* after 3-6 weeks of initial exposure to HIV.
- iii) Initially, sudden marked reduction in CD4⁺ T cells (helper T cells) followed by return to normal levels.

TABLE 13.4: Major Abnormalities in Immune System in AIDS.

1. T CELL ABNORMALITIES

- (i) Lymphopenia with depletion of CD4⁺ T cells; reversal of CD4 to CD8 cell ratio
- (ii) Susceptibility to opportunistic infections
- (iii) Susceptibility to neoplasms
- (iv) Decreased delayed type hypersensitivity
- (v) Decreased proliferative response to mitogens and soluble antigens
- (vi) Decreased specific cytotoxicity
- (vii) Decreased production of interleukin 2

2. B CELL ABNORMALITIES

- (i) Decreased Ig production
- (ii) Polyclonal activation
- (iii) Hypergammaglobulinaemia
- (iv) Circulating immune complexes

3. ABNORMALITIES OF MONOCYTE-MACROPHAGE CELL LINE

- (i) Decreased chemotaxis
- (ii) Decreased cytotoxicity

4. NK CELL ABNORMALITIES

- Decreased cytotoxicity

iv) Rise in CD8⁺ T cells (HIV-specific cytotoxic T cells).
v) Appearance of self-limited non-specific *acute viral illness* (flu-like or infectious mononucleosis-like) in 50-70% of adults within 3-6 weeks of initial infection (CDC group 1). Manifestations include: sore throat, fever, myalgia, skin rash, and sometimes aseptic meningitis. These symptoms resolve spontaneously in 2-3 weeks.

2. Middle chronic phase (10-12 years). The initial acute seroconversion illness is followed by a phase of competition between HIV and the host immune response as under:

i) *Viraemia* due to viral replication in the lymphoid tissue continues which is initially not as high but with passage of time viral load increases due to crumbling host defenses.

TABLE 13.5: Natural History of HIV Infection.

PHASE	EARLY, ACUTE	MIDDLE, CHRONIC	FINAL, CRISIS
<i>Period after infection</i>	3-6 weeks	10 to 12 years	Any period up to death
<i>CDC category</i>	Group I: Acute HIV syndrome	Group II: Asymptomatic infection Group III: PGL	Group IV: 5 Subgroups: A: Constitutional symptoms B: Neurologic disease C: Secondary infection D: Secondary neoplasms E: Other conditions
<i>Plasma viraemia</i>	High	Moderate	High
<i>CD4 counts</i>	Suddenly reduced	Moderate loss	Severely reduced (< 200/μl)

(CDC = Centres for Disease Control, Atlanta, USA; PGL = Persistent generalised lymphadenopathy).

- ii) Chronic stage depending upon host immune system may continue as long as 10 years.
- iii) CD 4+ T cells continue to proliferate but net result is moderate fall in CD4+ T cell counts.
- iv) Cytotoxic CD8+ T cell count remains high.
- v) Clinically, it is a stage of *latency*. The patient may be asymptomatic (CDC group II), or may develop mild constitutional symptoms and persistent generalised lymphadenopathy (CDC group III).

3. Final crisis phase. This phase is characterised by profound immunosuppression and onset of full-blown AIDS and has the following features:

- i) Marked increase in *viraemia*.
- ii) The time period between HIV infection developing into chronic phase followed by full-blown AIDS may last 7-10 years and culminates in death.
- iii) CD 4+ T cells are markedly reduced (below 200 per μ l).
- iv) Clinically, the features of the final phase are categorised into following 5 subgroups (CDC group IV) (Fig. 13.4):

Subgroup A: *Constitutional disease* characterised by fever of more than one month duration, weight loss of

more than 10% of body weight, and chronic diarrhoea lasting more than one month.

Subgroup B: *Neurologic disease* present in majority of cases and include viral meningoencephalitis, aseptic meningitis, peripheral neuropathy, AIDS-dementia complex.

Subgroup C: *Secondary opportunistic infections* such as fungal, viral, bacterial and parasitic.

Subgroup D: *Secondary neoplasms* such as Kaposi's sarcoma, Non-Hodgkin's lymphoma (Burkitt's, immunoblastic), Hodgkin's lymphoma, primary CNS lymphoma.

Subgroup E: This recent subgroup includes < 200 CD 4+ T lymphocytes/ μ l, pulmonary tuberculosis and recurrent pneumonia.

The average survival after the onset of full-blown AIDS is 18-24 months.

PATHOLOGIC CHANGES. The morphological changes in AIDS are non-specific and variable depending upon the stage of the disease. In general, the autopsy findings include the following (Fig. 13.4):

1. *Lymphoid tissues* (lymph nodes, spleen, thymus and bone marrow) show predominant changes *viz.*:

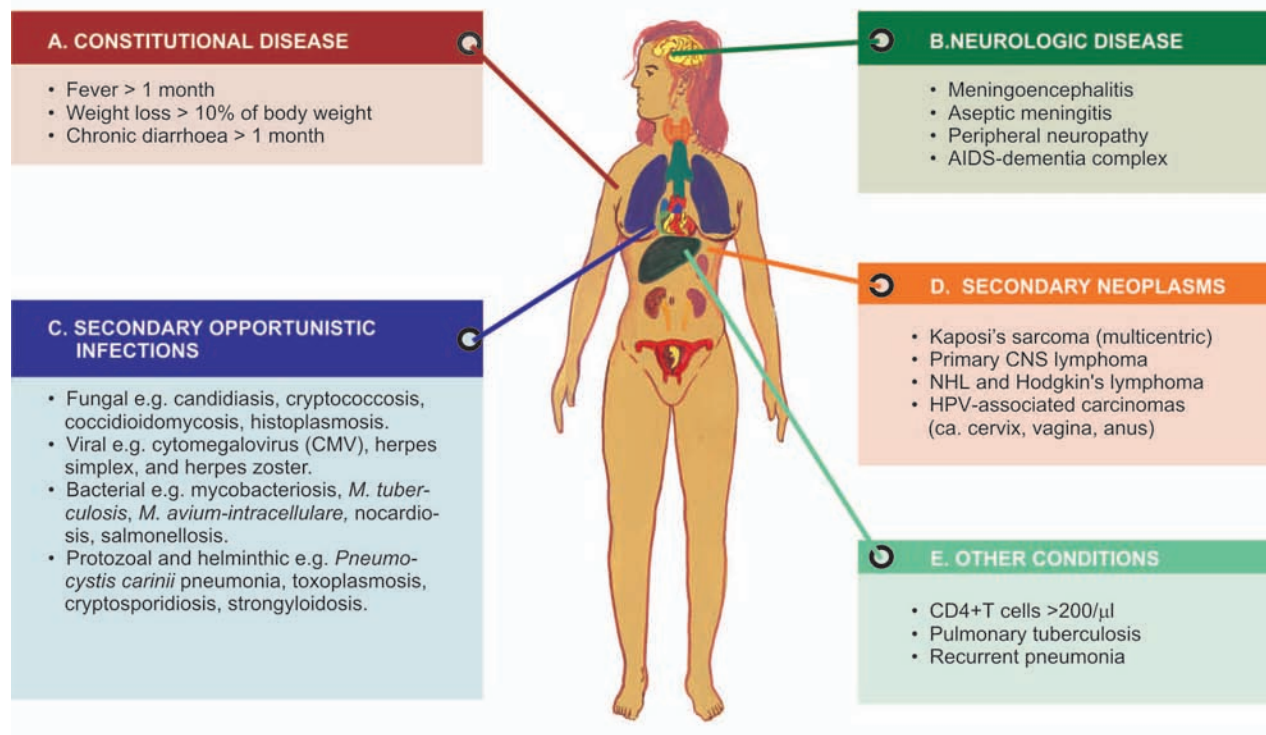


FIGURE 13.4

Major clinical manifestations of full-blown AIDS.

- In early stage, there is B cell hyperplasia in the form of nonspecific follicular hyperplasia and plasmacytosis in the medulla of the lymph nodes.
 - The full blown AIDS is characterised by universal lymphoid cell depletion, which, due to immunosuppression, may reveal evidence of opportunistic infections. Malignant lymphomas and other T cell neoplasms may occur.
2. Widespread opportunistic infections.
 3. AIDS-associated cancers e.g. HPV-associated cancers such as carcinoma of uterine cervix, vagina, anus.
 4. Neurological manifestations includes AIDS-associated dementia.
 5. AIDS associated nephropathy characterised by focal segmental glomerulosclerosis.

LABORATORY DIAGNOSIS OF AIDS. The investigations of a suspected case of AIDS include *initial testing for antibodies against HIV by ELISA and confirmation by Western blot or immunofluorescence test.*

The various laboratory tests currently used in research and clinical laboratories are categorised into 2 groups:

1. Specific tests:

- i) Serologic tests by ELISA, Western blot and immunofluorescence test
- ii) Antigen detection tests using envelope and core proteins of HIV by recombinant DNA techniques
- iii) Virus isolation and culture in neoplastic T cell line
- iv) Polymerase chain reaction (PCR).

2. Indirect tests:

- i) CD4 and CD8 cell counts; reversal of CD4 to CD8 cell ratio
- ii) Lymphopenia
- iii) Lymph node biopsy
- iv) Platelet count revealing thrombocytopenia
- v) Increased β -2 microglobulin levels.

HYPERSENSITIVITY REACTIONS (IMMUNOLOGIC TISSUE INJURY)

A state of balance in the immune responses (humoral or cell-mediated) is essential for protection against endogenous and exogenous antigens. *Hypersensitivity is defined as a state of exaggerated immune response to an antigen.* The lesions of hypersensitivity (immunologic tissue injury) are produced due to interaction between antigen and product of the immune response.

Depending upon the *rapidity* and *duration* of the immune response, two distinct forms of hypersensitivity reactions are recognised:

1. Immediate type in which on administration of antigen, the reaction occurs immediately (within seconds to minutes). Immune response in this type is mediated largely by *humoral antibodies*. Immediate type of hypersensitivity is further of 3 types—*type I, II and III.*

2. Delayed type in which the reaction is slower in onset and develops within 24-48 hours and the effect is prolonged. It is mainly mediated by *cellular response*. *Type IV reaction* is the delayed hypersensitivity reaction.

The mechanisms and examples of immunologic tissue injury by the 4 types of hypersensitivity reactions are summarised in Table 13.6.

Type I: Anaphylactic, Atopic Reaction

Anaphylaxis is the opposite of prophylaxis. It is defined as a state of rapidly developing immune response to an antigen to which the individual is previously sensitised.

The response is mediated by *humoral antibodies of IgE type or reagin antibodies*. IgE antibodies sensitise basophils of peripheral blood or mast cells of tissues leading to release of pharmacologically-active substances called *anaphylactic mediators*. These substances are histamine, serotonin, vasoactive intestinal peptide (VIP), chemotactic factors of anaphylaxis for neutrophils and eosinophils, leukotrienes B_4 and D_4 , prostaglandins (thromboxane A_2 , prostaglandin D_2 and E_2) and platelet activating factor. The effects of these agents are:

- increased vascular permeability;
- smooth muscle contraction;
- early vasoconstriction followed by vasodilatation;
- shock;
- increased gastric secretion; and
- increased nasal and lacrimal secretions.

The clinical examples of anaphylaxis may be of 2 types: *systemic or local.*

1. Examples of **systemic anaphylaxis** are:

- i) administration of antisera e.g. anti-tetanus serum (ATS);
- ii) administration of drugs e.g. penicillin; and
- iii) sting by wasp or bee.

The clinical features of systemic anaphylaxis include itching, erythema, contraction of respiratory bronchioles, diarrhoea, pulmonary oedema, pulmonary haemorrhage, shock and death.

2. Examples of **local anaphylaxis** are:

- i) hay fever (seasonal allergic rhinitis) due to pollen sensitisation of conjunctiva and nasal passages;

TABLE 13.6: Mechanisms of Immunologic Tissue Injury.

TYPE	MECHANISM	EXAMPLES
Type I (<i>Anaphylactic, atopic</i>)	Humoral Abs of IgE type → basophils/mast cells sensitised → release of anaphylactic mediators	i. Systemic anaphylaxis (administration of antisera and drugs, stings) ii. Local anaphylaxis (hay fever, bronchial asthma, food allergy, cutaneous, angioedema)
Type II (<i>Cytotoxic</i>)	i. Cytotoxic auto- and iso-antibodies to blood cells ii. Cytotoxic Abs to tissue components iii. Ab-dependent cell-mediated cytotoxicity (ADCC)	i. Autoimmune haemolytic anaemia, transfusion reactions, erythroblastosis foetalis, ITP, leucopenia, drug-induced ii. Myasthenia gravis, male sterility iii. Tumour cells, parasites
Type III (<i>Immune-complex</i>)	i. Local (Arthus reaction) Ag-Ab complexes ii. Systemic (circulating) Ag-Ab complexes or serum sickness	i. Injection of ATS, farmer's lung ii. Glomerulonephritis, collagen diseases, Goodpasture's syndrome, arthritis, uveitis, skin diseases
Type IV (<i>Cell-mediated</i>)	i. Classical delayed hypersensitivity mediated by CD4 + T cells ii. T cell mediated cytotoxicity by CD8+ T cells	i. Tuberculin reaction, tuberculosis, tuberculoid leprosy, typhoid, contact dermatitis ii. Virus-infected cells, tumour cells, transplant rejection

ii) bronchial asthma due to allergy to inhaled allergens like house dust;

iii) food allergy to ingested allergens like fish, cow's milk etc;

iv) cutaneous anaphylaxis due to contact of antigen with skin characterised by urticaria, wheal and flare; and

v) angioedema, an autosomal dominant inherited disorder characterised by laryngeal oedema, oedema of eyelids, lips, tongue and trunk.

Local anaphylaxis is common, affecting about 10% of population. About 50% of these conditions are familial with genetic predisposition and therefore also called *atopic reactions*.

Type II: Cytotoxic Reaction

Cytotoxic reactions are defined as those reactions which cause injury to the cell by combining humoral antibodies with cell surface antigens; blood cells being affected more commonly.

Three types of mechanisms are involved in mediating cytotoxic reactions:

1. Cytotoxic antibodies to blood cells. This mechanism involves direct cytolysis of blood cells (red blood cells, leucocytes and platelets) by combining the cell surface antigen with IgG or IgM class antibodies. In the process, complement system is activated resulting in injury to the cell membrane. The cell surface is made susceptible to phagocytosis due to coating or opsonisation from serum factors or opsonins.

Examples of cytotoxicity by this mechanism are as under:

i) *Autoimmune haemolytic anaemia* in which the red cell injury is brought about by autoantibodies reacting with antigens present on red cell surface. Antiglobulin test (direct Coombs' test) is employed to detect the antibody on red cell surface (Chapter 33).

ii) *Transfusion reactions* due to incompatible or mismatched blood transfusion.

iii) Haemolytic disease of the newborn (*erythroblastosis foetalis*) in which the foetal red cells are destroyed by maternal isoantibodies crossing the placenta.

iv) *Idiopathic thrombocytopenic purpura* (ITP) is the destruction of platelets by autoantibodies reacting with surface components of normal platelets.

v) *Leucopenia with agranulocytosis* may be caused by autoantibodies to leucocytes causing their destruction.

vi) *Drug-induced cytotoxic antibodies* are formed in response to administration of certain drugs like penicillin, methyl dopa, rifampicin etc. The drugs or their metabolites act as haptens binding to the surface of blood cells to which the antibodies combine, bringing about destruction of cells.

2. Cytotoxic antibodies to tissue components. Cellular injury may be brought about by autoantibodies reacting with some components of tissue cells in certain diseases.

Examples are as under:

i) In *Graves' disease* (primary hyperthyroidism), thyroid autoantibody is formed which reacts with the TSH receptor to cause hyperfunction and proliferation.

ii) In *myasthenia gravis*, antibody to acetylcholine receptors of skeletal muscle is formed which blocks the neuromuscular transmission at the motor end-plate, resulting in muscle weakness.

iii) In *male sterility*, antisperm antibody is formed which reacts with spermatozoa and causes impaired motility as well as cellular injury.

More recently, however, Graves' disease has been categorised separately under *type V hypersensitivity reaction (stimulatory type)*. TSH receptor on thyroid cell membrane reacts with long-acting thyroid stimulating (LATS) autoantibody which, in turn, brings about chemical overactivity (stimulatory type) of thyroid gland (i.e. hyperthyroidism).

3. Antibody-dependent cell-mediated cytotoxicity (ADCC). Cytotoxicity by this mechanism is mediated by leucocytes like monocytes, neutrophils, eosinophils and NK cells. The antibodies involved are mostly IgG class. The cellular injury occurs by lysis of antibody-coated target cells through Fc receptors on leucocytes.

The *examples* of target cells killed by this mechanism are tumour cells, parasites etc.

Type III: Immune Complex Reaction

Type III reactions result from formation of immune complexes by direct antigen-antibody (Ag-Ab) combination as a result of which the complement system gets activated causing cell injury.

Two types of antigens can cause immune complex-mediated tissue injury:

1. *Exogenous antigens* such as infectious agents (bacteria, viruses, fungi, parasites); certain drugs and chemicals.
2. *Endogenous antigens* such as blood components (immunoglobulins, tumour antigens) and antigens in cells and tissues (nuclear antigens in SLE).

Depending upon the distribution and location of antigens, type III reactions are of 2 types:

1. Local: Arthus reaction. It is a localised inflammatory reaction, usually an immune complex vasculitis of skin, in an individual with circulating antibody. Large immune complexes are formed due to excess of antibodies which precipitate locally in the vessel wall causing fibrinoid necrosis.

Examples of local immune complex disease are:

- i) injection of antitetanus serum; and
- ii) farmer's lung in which there is allergic alveolitis in response to bacterial antigen from mouldy hay.

2. Systemic: Circulating immune complex disease or serum sickness. The steps involved in cell injury by circulating immune complexes are as follows:

- i) After the antigen is introduced into the circulation, it initiates formation of antibodies which react with antigen to form circulating Ag-Ab complexes.

ii) These complexes are then deposited at different tissue sites containing basement membrane exposed to circulating blood e.g. basement membrane of renal glomeruli, lungs, choroid plexus of brain, uveal tract of eye, synovial membrane of joints.

iii) Following deposition of Ag-Ab complexes in the tissues, there is acute inflammatory reaction and activation of the complement system with elaboration of biologically-active compounds such as chemotactic factors, vasoactive amines and anaphylatoxins. The immune complexes are phagocytosed by leucocytes drawn at the site by chemotactic factors.

Examples of circulating immune complex diseases are as under:

- i) Various forms of glomerulonephritis e.g. acute glomerulonephritis, membranous glomerulonephritis, glomerulonephritis in SLE (lupus nephritis).
- ii) Collagen diseases e.g. SLE, polyarteritis nodosa, scleroderma, rheumatoid arthritis, Sjögren's syndrome, dermatomyositis.
- iii) Goodpasture's syndrome caused by an antibody, common to pulmonary as well as renal basement membrane, and produces glomerulonephritis associated with pulmonary haemorrhage.
- iv) Arthritis occurring transiently during infections.
- v) Uveitis.
- vi) Skin diseases.

Type IV: Cell-Mediated Reaction

This type of hypersensitivity is mediated by specifically-sensitised T lymphocytes produced in the cell-mediated immune response.

Cell-mediated reactions are of 2 main types:

1. Classical delayed hypersensitivity. This is mediated by specifically sensitised CD4⁺ T cell subpopulation on contact with antigen. These cells possess surface receptors which bind to the antigen, resulting in cell injury characterised by slowly developing inflammatory response and hence the name delayed hypersensitivity.

The classical *example* of delayed hypersensitivity is the tuberculin reaction. On intradermal injection of tuberculoprotein (PPD), an unsensitised individual develops no response (tuberculin negative). On the other hand, a person who has developed cell-mediated immunity to tuberculoprotein as a result of BCG immunisation or has been exposed to tuberculous infection develops typical delayed inflammatory reaction, reaching its peak in 48 hours (tuberculin positive), after which it subsides slowly. *Microscopically*, mononuclear inflammatory cells in and around small blood vessels and oedema are seen.

Other examples of delayed hypersensitivity response are tuberculosis, tuberculoid leprosy, typhoid fever, and contact dermatitis due to allergic response to a number of chemicals and allergens.

2. T cell-mediated cytotoxicity. CD8⁺ subpopulation of T lymphocytes are the cytotoxic T cells (T_{CTL}) and are generated in response to antigens like virus-infected cells, tumour cells and incompatible transplanted tissue or cells.

AUTOIMMUNE DISEASES

Autoimmunity is a state in which the body's immune system fails to distinguish between 'self' and 'non-self' and reacts by formation of autoantibodies against one's own tissue antigens. In other words, there is loss of tolerance to one's own tissues; *autoimmunity is the opposite of immune tolerance.*

Immune tolerance is a normal phenomenon present since foetal life and is defined as the ability of an individual to recognise self tissue and antigens. Normally, the immune system of the body is able to distinguish self from non-self antigens by the following mechanisms:

1. *Clonal elimination.* According to this theory, during embryonic development, T cells maturing in the thymus acquire the ability to distinguish self from non-self. These T cells are then eliminated by apoptosis for the tolerant individual.
2. *Concept of clonal anergy.* According to this mechanism, T lymphocytes which have acquired the ability to distinguish self from non-self are not eliminated but instead become non-responsive and inactive.
3. *Suppressor T cells.* According to this mechanism, the tolerance is achieved by a population of specific suppressor T cells which do not allow the antigen-responsive cells to proliferate and differentiate.

PATHOGENESIS OF AUTOIMMUNITY

The mechanisms by which the immune tolerance of the body is broken causes autoimmunity. These mechanisms may be immunological, genetic, and microbial, all of which may be interacting.

1. Immunological factors. Failure of immunological mechanisms of tolerance initiates autoimmunity. These mechanisms are as follows:

- i) *Polyclonal activation of B cells.* B cells may be directly activated by stimuli such as infection with microorganisms and their products leading to bypassing of T cell tolerance.
- ii) *Generation of self-reacting B cell clones* may also lead to bypassing of T cell tolerance.

- iii) *Decreased T suppressor and increased T helper cell activity.* Loss of T suppressor cell and increase in T helper cell activities may lead to high levels of auto-antibody production by B cells contributing to auto-immunity.

- iv) *Fluctuation of anti-idiotypic network control* may cause failure of mechanisms of immune tolerance.

- v) *Sequestered antigen released from tissues.* 'Self-antigen' which is completely sequestered may act as 'foreign-antigen' if introduced into the circulation later. For example, in trauma to the testis, there is formation of anti-sperm antibodies against spermatozoa; similar is the formation of autoantibodies against lens crystallin.

2. Genetic factors. There is evidence in support of genetic factors in the pathogenesis of autoimmunity as under:

- i) There is increased expression of *Class II HLA antigens* on tissues involved in autoimmunity.
- ii) There is increased *familial incidence* of some of the autoimmune disorders.

3. Microbial factors. Infection with microorganisms, particularly viruses (e.g. EBV infection), and less often bacteria (e.g. streptococci, *Klebsiella*) and mycoplasma, has been implicated in the pathogenesis of autoimmune diseases. However, a definite evidence in support is lacking.

TYPES AND EXAMPLES OF AUTOIMMUNE DISEASES

Depending upon the type of autoantibody formation, the autoimmune diseases are broadly classified into 2 groups:

1. Organ specific diseases. In these, the autoantibodies formed react specifically against an organ or target tissue component and cause its chronic inflammatory destruction. The tissues affected are endocrine glands (thyroid, pancreatic islets of Langerhans, adrenal cortex), alimentary tract, blood cells and various other tissues and organs.

2. Non-organ specific diseases. These are diseases in which a number of autoantibodies are formed which react with antigens in many tissues and thus cause systemic lesions. The examples of this group are various systemic collagen diseases.

However, a few autoimmune diseases overlap between these two main categories.

Based on these 2 main groups, a comprehensive list of autoimmune (or collagen) diseases is presented in Table 13.7. Some of the systemic autoimmune diseases (*marked with astrix*) are discussed in this chapter while others from both the groups are described later in the relevant chapters.

TABLE 13.7: Autoimmune Diseases.

NON-ORGAN SPECIFIC (SYSTEMIC)	ORGAN SPECIFIC (LOCALISED)
1. Systemic lupus erythematosus* 2. Rheumatoid arthritis 3. Scleroderma (Progressive systemic sclerosis)* 4. Polymyositis-dermatomyositis* 5. Polyarteritis nodosa (PAN) 6. Sjögren's syndrome* 7. Reiter's syndrome* 8. Mixed connective tissue disease*	1. ENDOCRINE GLANDS (i) Hashimoto's (autoimmune) thyroiditis (ii) Graves' disease (iii) Insulin-dependent diabetes mellitus (iv) Idiopathic Addison's disease 2. ALIMENTARY TRACT (i) Autoimmune atrophic gastritis in pernicious anaemia (ii) Ulcerative colitis (iii) Crohn's disease 3. BLOOD CELLS (i) Autoimmune haemolytic anaemia (ii) Autoimmune thrombocytopenia 4. OTHERS (i) Myasthenia gravis (ii) Autoimmune orchitis (iii) Autoimmune encephalomyelitis (iv) Goodpasture's syndrome (v) Primary biliary cirrhosis (vi) Lupoid hepatitis (vii) Membranous glomerulonephritis (viii) Autoimmune skin diseases

*Diseases discussed in this chapter.

Systemic Lupus Erythematosus (SLE)

SLE is the classical example of systemic autoimmune or collagen diseases. The disease derives its name 'lupus' from the Latin word meaning 'wolf' since initially this disease was believed to affect skin only and eat away skin like a wolf. However, now 2 forms of lupus erythematosus are described:

1. Systemic or disseminated form is characterised by acute and chronic inflammatory lesions widely scattered in the body and there is presence of various nuclear and cytoplasmic autoantibodies in the plasma.

2. Discoid form is characterised by chronic and localised skin lesions involving the bridge of nose and adjacent cheeks without any systemic manifestations. Rarely, discoid form may develop into disseminated form.

ETIOLOGY. The exact etiology of SLE is not known. However, autoantibodies against nuclear and cytoplasmic components of the cells are demonstrable in plasma by immunofluorescence tests in almost all cases of SLE. Some of the important *antinuclear antibodies* (ANAs) or antinuclear factors (ANFs) against different nuclear antigens are as under:

i) *Antinuclear antibodies* (ANA) are the antibodies against common nuclear antigen that includes DNA as well as RNA.

ii) *Antibodies to double-stranded (anti-dsDNA) or single-stranded DNA (anti-ssDNA)* is the most specific, present in half the cases of SLE.

iii) *Anti-Smith antigen antibodies (anti-SmAg)*, in which antibodies appear against Smith antigen which is part of ribonucleoproteins.

iv) *Anti-ribonucleoproteins (anti-RNP)* are not specific for SLE but are seen more often in Sjögren's syndrome.

v) *Anti-histone antibodies*, seen particularly in cases of drug-induced SLE.

vi) *Anti-nucleolar antibodies* to nucleolar antigens.

vii) *Antiphospholipid antibodies or lupus anticoagulant* are quite specific for SLE and are responsible for thrombotic complications in cases of SLE.

The source of these autoantibodies as well as hypergammaglobulinaemia in SLE is the **polyclonal activation of B cells** brought about by following derangements:

1. *Immunologic factors.* These include:

- i) an inherited defect in B cells;
- ii) stimulation of B cells by micro-organisms;
- iii) T helper cell hyperactivity; and
- iv) T suppressor cell defect.

2. *Genetic factors.* Genetic predisposition to develop autoantibodies to nuclear and cytoplasmic antigens in SLE is due to the immunoregulatory function of class II HLA genes implicated in the pathogenesis of SLE.

3. *Other factors.* Various other factors express the genetic susceptibility of an individual to develop clinical disease. These factors are:

- i) certain drugs e.g. penicillamine D;
- ii) certain viral infections e.g. EBV infection; and
- iii) certain hormones e.g. oestrogen.

PATHOGENESIS. The autoantibodies formed by any of the mechanisms explained above are the mediators of tissue injury in SLE. Two types of immunologic tissue injury can occur in SLE:

1. *Type II hypersensitivity* is characterised by formation of autoantibodies against blood cells (red blood cells, platelets, leucocytes) and results in haematologic derangement in SLE.

2. *Type III hypersensitivity* is characterised by antigen-antibody complex (commonly DNA-anti-DNA antibody; sometimes Ig-anti-Ig antibody complex) which is deposited at sites such as renal glomeruli, walls of small blood vessels etc.

LE CELL PHENOMENON. This was the first diagnostic laboratory test described for SLE. The test is based on the principle that ANAs cannot penetrate the intact cells and thus cell nuclei should be exposed to bind them with the ANAs. The binding of exposed nucleus with ANAs results in homogeneous mass of nuclear chromatin material which is called *LE body* or *haematoxylin body*.

LE cell is a phagocytic leucocyte, commonly polymorphonuclear neutrophil, and sometimes a monocyte, which engulfs the homogeneous nuclear material of the injured cell. For demonstration of LE cell phenomenon *in vitro*, the blood sample is traumatised to expose the nuclei of blood leucocytes to ANAs. This results in binding of denatured and damaged nucleus with ANAs. The ANA-coated denatured nucleus is chemotactic for phagocytic cells.

■ If this mass is engulfed by a neutrophil, displacing the nucleus of neutrophil to the rim of the cell, it is called *LE cell* (Fig. 13.5,A).

■ If the mass, more often an intact lymphocyte, is phagocytosed by a monocyte, it is called *Tart cell* (Fig. 13.5,B).

LE cell test is positive in 70% cases of SLE while newer and more sensitive immunofluorescence tests for autoantibodies are positive in almost 100% cases of SLE. A few other conditions may also show positive LE test e.g. rheumatoid arthritis, lupoid hepatitis, penicillin sensitivity etc.

PATHOLOGIC CHANGES. The manifestations of SLE are widespread in different visceral organs as well as show erythematous cutaneous eruptions. The

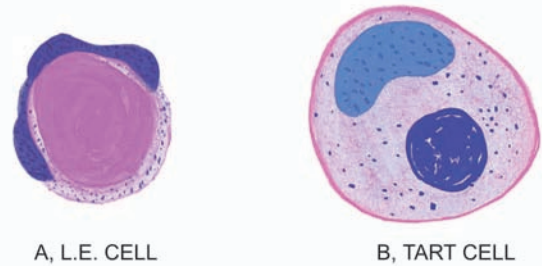


FIGURE 13.5

LE cell phenomenon. A. *Typical LE cell.* The rounded mass of amorphous nuclear material (LE body) has displaced the lobes of neutrophil to the rim of the cell. B. *Tart cell.* A lymphocyte with intact nuclear structure phagocytosed by monocyte, the nucleus of which is compressed.

principal lesions are renal, vascular, cutaneous and cardiac; other organs and tissues involved are serosal linings (pleuritis, pericarditis); joints (synovitis); spleen (vasculitis); liver (portal triaditis); lungs (interstitial pneumonitis, fibrosing alveolitis), CNS (vasculitis) and in blood (autoimmune haemolytic anaemia, thrombocytopaenia).

Microscopically, the characteristic lesion in SLE is *fibrinoid necrosis* which may be seen in the connective tissue, beneath the endothelium in small blood vessels, under the mesothelial lining of pleura and pericardium, under the endothelium in endocardium, or under the synovial lining cells of joints.

Table 13.8 summarises the morphology of lesions in different organs and tissues in SLE.

CLINICAL FEATURES. SLE, like most other autoimmune diseases, is more common in women in their 2nd to 3rd decades of life. As obvious from Table 13.8, SLE is a multisystem disease and thus a wide variety of clinical features may be present. A typical full-fledged case of SLE has the following characteristics:

- butterfly-like erythematous rash on skin of face;
- fever;
- painful joints;
- chest pain (due to pleuritis and pericarditis);
- renal involvement (proteinuria, haematuria, casts);
- haematologic derangements (haemolytic anaemia, thrombocytopenia, leucopenia); and
- neurologic disorders (seizures, psychosis).

The disease usually runs a long course of flare-ups and remissions; renal failure is the most frequent cause of death.

Scleroderma (Progressive Systemic Sclerosis)

Just like SLE, scleroderma was initially described as a skin disease characterised by progressive fibrosis. But now, 2 main types are recognised:

TABLE 13.8: Morphology of Major Lesions in SLE.

ORGANS (MAIN LESION)	MORPHOLOGIC FEATURES
1. <i>Renal lesions</i> (<i>Lupus nephritis</i>)	EM-shows large deposits in mesangium, subepithelial or subendothelial. IM-shows granular deposits of immune complex (IgG and C3) on capillaries, mesangium and tubular basement membranes. Six patterns: i. Minimal disease : LM shows no change ii. Mesangial GN : LM shows increase in matrix; mesangial hypercellularity. iii. Focal proliferative GN : LM shows focal (parts of 50% glomeruli) endothelial and mesangial cell proliferation; infiltration with neutrophils. iv. Diffuse proliferative GN : LM shows all glomeruli affected; proliferation of endothelial, mesangial and epithelial cells; epithelial crescents. v. Membranous lupus GN : LM shows diffuse basement membrane thickening; features of nephrotic syndrome. vi. Sclerosing nephropathy : LM shows hyalinisation of whole of glomeruli.
2. <i>Small blood vessels</i> (<i>Acute necrotising vasculitis</i>)	Affects all tissues; commonly skin and muscles involved; LM shows fibrinoid deposits in the vessel wall; perivascular infiltrate of mononuclear cells.
3. <i>Cutaneous lesions</i> (<i>Erythematous eruptions</i>)	Butterfly area on nose and cheek; LM shows liquefactive degeneration of basal layer of epidermis; oedema at dermoepidermal junction; acute necrotising vasculitis in dermis; IM shows immune complex deposits (IgG and C3) at dermo-epidermal junction.
4. <i>Cardiac lesions</i> (<i>Libman-Sacks endocarditis</i>)	Vegetations on mitral and tricuspid valves, may extend to mural endocardium, chordae tendineae; LM of vegetations shows fibrinoid material, necrotic debris, inflammatory cells, haematoxylin bodies may be present; connective tissue of endocardium and myocardium may show focal inflammation and necrotising vasculitis.

(EM = electron microscopy, LM = light microscopy; IM = immunofluorescence microscopy; GN = glomerulonephritis).

1. *Diffuse scleroderma* in which the skin shows widespread involvement and may progress to involve visceral structures.

2. *CREST syndrome* of progressive systemic sclerosis characterised by Calcinosis (C), Raynaud's phenomenon (R), Esophageal hypomotility (E), Sclerodactyly (S) and Telangiectasia (T).

ETIOPATHOGENESIS. The etiology of this disease is not known. However, antinuclear antibodies are detected in majority of cases of systemic sclerosis. Immunologic mechanisms have been implicated in the pathogenesis of lesions in systemic sclerosis which finally cause activation of fibroblasts. The *immune mechanisms* leading to stimulation of fibroblasts may act in the following ways:

1. *Elaboration of cytokines* such as by fibroblast growth factor and chemotactic factors by activated T cells and macrophages.
2. *Endothelial cell injury* due to cytotoxic damage to endothelium from autoantibodies or antigen-antibody

complexes. This results in aggregation and activation of platelets which increases vascular permeability and stimulates fibroblastic proliferation.

PATHOLOGIC CHANGES. Disseminated visceral involvement as well as cutaneous lesions are seen in systemic sclerosis.

1. Skin changes. Skin is involved diffusely, beginning distally from fingers and extending proximally to arms, shoulders, neck and face. In advanced stage, the fingers become claw-like and face mask-like.

Microscopically, changes are progressive from early to late stage.

■ *Early stage* shows oedema and degeneration of collagen. The small-sized blood vessels are occluded and there is perivascular infiltrate of mononuclear cells.

■ *Late stage* reveals thin and flat epidermis. Dermis is largely replaced by compact collagen and there is hyaline thickening of walls of dermal blood vessels.

In advanced cases subcutaneous calcification may occur.

2. Kidney changes. Involvement of kidneys is seen in majority of cases of systemic sclerosis. The lesions are prominent in the walls of interlobular arteries which develop changes resembling malignant hypertension. There is thickening of tunica intima due to concentric proliferation of intimal cells and fibrinoid necrosis of vessel wall.

3. Smooth muscle of GIT. Muscularis of the alimentary tract, particularly oesophagus, is progressively atrophied and replaced by fibrous tissue.

4. Skeletal muscle. The interstitium of skeletal muscle shows progressive fibrosis and degeneration of muscle fibres with associated inflammatory changes.

5. Cardiac muscle. Involvement of interstitium of the heart may result in heart failure.

6. Lungs. Diffuse fibrosis may lead to contraction of the lung substance. There may be epithelium-lined honey-combed cysts of bronchioles.

7. Small arteries. The lesions in small arteries show endarteritis due to intimal proliferation and may be the cause for Raynaud's phenomenon.

CLINICAL FEATURES. Systemic sclerosis is more common in middle-aged women. The clinical manifestations include:

- claw-like flexion deformity of hands;
- Raynaud's phenomenon;
- oesophageal fibrosis causing dysphagia and hypomotility;
- malabsorption syndrome;
- respiratory distress;
- malignant hypertension;
- pulmonary hypertension; and
- biliary cirrhosis.

Polymyositis-Dermatomyositis

As the name suggests, this disease is a combination of symmetric muscle weakness and skin rash.

ETIOPATHOGENESIS. The exact cause of the disease is unknown. However, antinuclear antibodies are detected in 25% of cases. Thus, an *immunologic hypothesis* has been proposed. The affected muscles are infiltrated by sensitised T lymphocytes of both T helper and T suppressor type which are considered to bring about inflammatory destruction of muscle. *Viral etiology* due to infection with coxsackie B virus has also been suggested.

PATHOLOGIC CHANGES. The skeletal muscles usually affected are of pelvis, shoulders, neck, chest and diaphragm.

Microscopically, vacuolisation and fragmentation of muscle fibres and numerous inflammatory cells are present. In late stage, muscle fibres are replaced by fat and fibrous tissue.

CLINICAL FEATURES. It is a multi-system disease characterised by:

- muscle weakness, mainly proximal;
- skin rash, typically with heliotropic erythema and periorbital oedema;
- dysphagia due to involvement of pharyngeal muscles;
- respiratory dysfunction; and
- association with deep-seated malignancies.

Sjögren's Syndrome

Sjögren's syndrome is characterised by the *triad* of dry eyes (*keratoconjunctivitis sicca*), dry mouth (*xerostomia*), and *rheumatoid arthritis*. The combination of the former two symptoms is called *sicca syndrome*.

ETIOPATHOGENESIS. Immune mechanisms have been implicated in the etiopathogenesis of lesions in Sjögren's syndrome. Antinuclear antibodies are found in about 90% of cases; test for rheumatoid factor is positive in 25% of cases. The lesions in lacrimal and salivary glands are mediated by T lymphocytes, B cells and plasma cells.

PATHOLOGIC CHANGES. *In early stage*, the lacrimal and salivary glands show periductal infiltration by lymphocytes and plasma cells, which at times may form lymphoid follicles (pseudolymphoma). *In late stage*, glandular parenchyma is replaced by fat and fibrous tissue. The ducts are also fibrosed and hyalinised.

CLINICAL FEATURES. The disease is common in women in 4th to 6th decades of life. It is clinically characterised by:

- Symptoms referable to eyes such as blurred vision, burning and itching.
- Symptoms referable to xerostomia such as fissured oral mucosa, dryness, and difficulty in swallowing.
- Symptoms due to glandular involvement such as enlarged and inflamed lacrimal gland (Mikulicz's syndrome is involvement of parotid alongwith lacrimal gland).

■ Symptoms due to *systemic involvement* referable to lungs, CNS and skin.

Reiter's Syndrome

This syndrome is characterised by *triad of arthritis, conjunctivitis and urethritis*. There may be mucocutaneous lesions on palms, soles, oral mucosa and genitalia. Antinuclear antibodies and RA factor are usually negative.

Mixed Connective Tissue Disease

This designation is applied to a syndrome having features of 3 collagen diseases—SLE, progressive systemic sclerosis, and dermatomyositis. High titers of antinuclear antibodies are detected. The clinical features are varied and consist of manifestations of diseases included. However, renal involvement is generally not seen and prognosis is good due to response to corticosteroid therapy.

TRANSPLANT REJECTION

According to the genetic relationship between donor and recipient, transplantation of tissues is classified into 4 groups:

1. *Autografts* are grafts in which the donor and recipient is the same individual.
2. *Isografts* are grafts between the donor and recipient of the same genotype.
3. *Allografts* are those in which the donor is of the same species but of a different genotype.
4. *Xenografts* are those in which the donor is of a different species from that of the recipient.

All types of grafts have been performed in human beings but xenografts have been found to be rejected invariably due to genetic disparity. Presently, surgical skills exist for skin grafts and for organ transplants such as kidney, heart, lungs, liver, pancreas, cornea and bone marrow. But most commonly practised are skin grafting, kidney and bone marrow transplantation. For any successful tissue transplant without immunological rejection, matched major histocompatibility locus antigens (HLA) between the donor and recipient are of paramount importance (see page 147). The greater the genetic disparity between donor and recipient in HLA system, the stronger and more rapid will be the rejection reaction.

Besides the rejection reaction, a peculiar problem occurring especially in bone marrow transplantation is graft-versus-host reaction (GVH). In humans, GVH reaction results when immunocompetent cells are transplanted to an immunodeficient recipient e.g. when

severe combined immunodeficiency is treated by bone marrow transplantation. The clinical features of GVH reaction include: fever, weight loss, anaemia, dermatitis, diarrhoea, intestinal malabsorption, pneumonia and hepatosplenomegaly. The intensity of GVH reaction depends upon the extent of genetic disparity between the donor and recipient.

Mechanisms of Graft Rejection

Except for autografts and isografts, an immune response against allografts is inevitable. The development of immunosuppressive drugs has made the survival of allografts in recipients possible. Rejection of allografts involves both cell-mediated and humoral immunity.

1. CELL-MEDIATED IMMUNE REACTIONS. These are mainly responsible for graft rejection and are mediated by T cells. The lymphocytes of the recipient on coming in contact with HLA antigens of the donor are sensitised in case of incompatibility. The sensitised T cells in the form of cytotoxic T cells (T_{CTL}) as well as by hypersensitivity reactions initiated by helper T cells attack the graft and destroy it.

2. HUMORAL IMMUNE REACTIONS. Currently, in addition to the cell-mediated immune reactions, a role for humoral antibodies in certain rejection reactions has been suggested. These include: *preformed circulating antibodies* due to pre-sensitisation of the recipient before transplantation e.g. by blood transfusions and previous pregnancies, or in *non-sensitised individuals* by complement dependent cytotoxicity, antibody-dependent cell-mediated cytotoxicity (ADCC) and antigen-antibody complexes.

Types of Rejection Reactions

Based on the underlying mechanism and time period, rejection reactions are classified into 3 types: hyperacute, acute and chronic.

1. HYPERACUTE REJECTION. Hyperacute rejection appears within minutes to hours of placing the transplant and destroys it. It is mediated by preformed humoral antibody against donor-antigen.

Grossly, hyperacute rejection is recognised by the surgeon soon after the vascular anastomosis of the graft is performed to the recipient's vessels. The organ becomes swollen, oedematous, haemorrhagic, purple and cyanotic rather than gaining pink colour.

Microscopically, the characteristics of Arthus reaction are present. There are numerous neutrophils around

dilated and obstructed capillaries which are blocked by fibrin and platelet thrombi. Small segments of blood vessel wall may become necrotic and there is necrosis of much of the transplanted organ. Small haemorrhages are common.

Cross-matching of the donor's lymphocytes with those of the recipient before transplantation has diminished the frequency of hyperacute rejection.

2. ACUTE REJECTION. This usually becomes evident within a few days to a few months of transplantation. Acute graft rejection may be mediated by cellular or humoral mechanisms.

Acute cellular rejection is more common and is characterised by extensive infiltration in the interstitium of the transplant by lymphocytes (mainly T cells), a few plasma cells, monocytes and a few polymorphs. There is damage to the blood vessels and there are foci of necrosis in the transplanted tissue. Acute humoral rejection appears due to poor response to immuno-

suppressive therapy. It is characterised by acute rejection vasculitis and foci of necrosis in small vessels. The mononuclear cell infiltrate is less marked as compared to acute cellular rejection and consists mostly of B lymphocytes.

3. CHRONIC REJECTION. Chronic rejection may follow repeated attacks of acute rejection or may develop slowly over a period of months to a year or so. The underlying mechanisms of chronic rejection may be immunologic or ischaemic. Patients with chronic rejection of renal transplant show progressive deterioration in renal function as seen by rising serum creatinine levels.

PATHOLOGIC CHANGES. In chronic rejection of transplanted kidney, the changes are intimal fibrosis, interstitial fibrosis and tubular atrophy. Renal allografts may develop glomerulonephritis by transmission from the host, or rarely may be *de novo* glomerulonephritis.



Infections and Infestations

INTRODUCTION

Microorganisms, namely bacteria, viruses, fungi and parasites, are present everywhere—in the soil, water, atmosphere and on the body surfaces, and are responsible for a large number of infectious diseases in human beings. Some microorganisms are distributed throughout the world while others are limited to certain geographic regions only. In general, tropical and under-developed countries are specially affected by infectious diseases than the developed countries. Certain *infectious diseases* are not so common in the developed world now but they continue to be major health problems in the under-developed countries e.g. tuberculosis, leprosy, typhoid fever, cholera, measles, pertussis, malaria, amoebiasis, pneumonia etc. *Vaccines* have been successful in controlling or eliminating some diseases e.g. smallpox, poliomyelitis, measles, pertussis etc. Insecticides have helped in controlling malaria to an extent. However, infections still rank very high *as a cause of death* in the world. Reasons for this trend are not difficult to seek:

- development of newer and antibiotic-resistant strains of microorganisms;
- administration of immunosuppressive therapy to patients with malignant tumours and transplanted organs making them susceptible to opportunistic infections;
- increasing number of patients reporting to hospital for different illnesses but instead many developing hospital-acquired infections, and
- lastly and more recently is the discovery in 1981 of previously unknown deadly disease i.e. acquired immunodeficiency syndrome (AIDS) caused by human immunodeficiency virus (HIV).

While talking of infective diseases, let us not forget the fact that many microorganisms may actually benefit mankind. Following is the range of *host-organism inter-relationship*, which may vary quite widely:

1. *Symbiosis* i.e. cooperative association between two dissimilar organisms beneficial to both.
2. *Commensalism* i.e. two dissimilar organisms living together benefitting one without harming the other.
3. *True parasitism* i.e. two dissimilar organisms living together benefitting the parasite but harming the host.

4. *Saprophytism* i.e. organisms living on dead tissues.

Besides the microorganisms, more recently a modified host protein present in the mammalian CNS has been identified called *prion protein*. Prions are transmissible agents just as infectious particles but lack nucleic acid. These agents are implicated in the etiology of spongiform encephalopathy, (including kuru), bovine spongiform encephalopathy (or mad cow disease) and Creutzfeldt-Jakob disease (associated with corneal transplantation). (Dr. Prusiner who discovered prion protein was awarded Nobel Prize in medicine in 1997).

Infectious diseases are the consequence of inter-relationship between disease-producing properties of microorganisms and host-defense capability against the invading organisms. Briefly, factors determining this host-microorganism relationship are given below:

Factors Relating to Infectious Agents

These are as under:

- i) **Mode of entry.** Microorganisms causing infectious disease may gain entry into the body by various routes e.g.

- through ingestion (external route);
- inoculation (parenteral method);
- inhalation (respiration);
- perinatally (vertical transmission);
- by direct contact (contagious infection); and
- by contaminated water, food, soil, environment or from an animal host (zoonotic infections).

- ii) **Spread of infection.** Microorganisms after entering the body may spread further through the phagocytic cells, blood vessels and lymphatics.

- iii) **Production of toxins.** Bacteria liberate toxins which have effects on cell metabolism. *Endotoxins* are liberated on lysis of the bacterial cell while *exotoxins* are secreted by bacteria and have effects at distant sites too.

- iv) **Virulence of organisms.** Many species and strains of organisms may have varying virulence e.g. the three strains of *C. diphtheriae* (*gravis*, *intermedius* and *mitis*) produce the same diphtherial exotoxin but in different amounts.

v) **Product of organisms.** Some organisms produce enzymes that help in spread of infections e.g. hyaluronidase by *Cl. welchii*, streptokinase by streptococci, staphylokinase and coagulase by staphylococci.

Factors Relating to Host

Microorganisms invade human body when defenses are not adequate. These factors include the following:

i) **Physical barrier.** A break in the continuity of the skin and mucous membranes allows the microorganisms to enter the body.

ii) **Chemical barrier.** Mucus secretions of the oral cavity and the alimentary tract and gastric acidity prevent bacterial colonisation.

iii) **Effective drainage.** The natural passages of the hollow organs like respiratory, gastrointestinal, urinary and genital system provide a way to drain the excretions effectively. Similarly, ducts of various glands are the conduits of drainage of secretions. Obstruction in any of these passages promotes infection.

iv) **Immune defense mechanisms.** These include the phagocytic leucocytes of blood (polymorphs and monocytes), phagocytes of tissues (mononuclear-phagocyte system) and the immune system as discussed in Chapter 10.

Some of the common diseases produced by pathogenic microorganisms are discussed below. Each group of microorganisms discussed here is accompanied by a list of diseases produced by them in a table. These lists of diseases are in no way complete but include only important and common examples. No attempts will be made to give details of organisms as that would mean repeating what is given in the textbooks of Microbiology. Instead, salient clinicopathologic aspects of these diseases are highlighted.

Methods of Identification

The organisms causing infections and parasitic diseases may be identified by routine H & E stained sections in many instances. However, confirmation in most cases requires either application of special staining techniques or by molecular biologic methods (Table 14.1). In addition, culture of lesional tissue should be carried out for species identification and drug sensitivity. Generally, the organism is looked for at the advancing edge of the lesion rather than in the necrotic centre.

DISEASES CAUSED BY BACTERIA, SPIROCHAETES AND MYCOBACTERIA

In order to gain an upper hand in human host, bacteria must resist early engulfment by neutrophils. They

TABLE 14.1: Methods of Identification of Microorganisms.

1. **Bacteria**
 - i. Gram stain: Most bacteria
 - ii. Acid fast stain: Mycobacteria, Nocardia
 - iii. Giemsa: Campylobacteria
2. **Fungi**
 - i. Silver stain: Most fungi
 - ii. Periodic acid-Schiff (PAS): Most fungi
 - iii. Mucicarmine: Cryptococci
3. **Parasites**
 - i. Giemsa: Malaria, Leishmania
 - ii. Periodic acid-Schiff: Amoebae
 - iii. Silver stain: Pneumocystis
4. **All classes including viruses**
 - i. Culture
 - ii. *In situ* hybridisation
 - iii. DNA analysis
 - iv. Polymerase chain reaction (PCR)

survive and damage the host in a variety of ways such as by generation of toxins (e.g. gas-forming anaerobes), by forming a slippery capsule that resists attachment to macrophages (e.g. pneumococci), by inhibition of fusion of phagocytic vacuoles with lysosomes (e.g. tubercle bacilli) etc.

Table 14.2 provides an abbreviated classification of bacterial diseases and their etiologic agents. A few common and important examples amongst these are discussed below.

ENTERIC FEVER

The term enteric fever is used to describe acute infection caused by *Salmonella typhi* (typhoid fever) or *Salmonella paratyphi* (paratyphoid fever). Besides these 2 salmonellae, *Salmonella typhimurium* causes food poisoning.

PATHOGENESIS. The typhoid bacilli are ingested through contaminated food or water. During the initial asymptomatic incubation period of about 2 weeks, the bacilli invade the lymphoid follicles and Peyer's patches of the small intestine and proliferate. Following this, the bacilli invade the blood stream causing bacteraemia, and the characteristic clinical features of the disease like continuous rise in temperature and 'rose spots' on the skin are observed. Immunological reactions (Widal's test) begin after about 10 days and peak titres are seen by the end of the third week. Eventually, the bacilli are localised in the intestinal lymphoid tissue (producing typhoid intestinal lesions), in the mesenteric lymph nodes (leading to haemorrhagic lymphadenitis), in the

TABLE 14.2: Diseases Caused by Bacteria, Spirochaetes and Mycobacteria.

DISEASE	ETIOLOGIC AGENT
1. Typhoid (enteric) fever	<i>Salmonella typhi</i>
2. Plague*	<i>Yersinia pestis</i>
3. Anthrax*	<i>Bacillus anthracis</i>
4. Whooping cough* (pertussis)	<i>Bordetella pertussis</i>
5. Chancroid	<i>Haemophilus ducreyi</i>
6. Granuloma inguinale*	<i>Calymmatobacterium donovani</i>
7. Gonorrhoea	<i>Neisseria gonorrhoeae</i>
8. Cholera	<i>Vibrio cholerae</i>
9. Shigellosis	<i>S. dysenteriae</i> , <i>S. flexneri</i> , <i>S. boydii</i> , <i>S. sonnei</i>
10. Brucellosis	<i>B. melitensis</i> , <i>B. abortus</i> , <i>B. suis</i> , <i>B. canis</i>
11. Diphtheria	<i>Corynebacterium diphtheriae</i>
12. Lobar pneumonia (Chapter 26)	<i>Streptococcus pneumoniae</i> , <i>Staphylococcus aureus</i> , <i>Haemophilus influenzae</i> , <i>Klebsiella pneumoniae</i>
13. Bronchopneumonia (Chapter 26)	<i>Staphylococci</i> , <i>Streptococci</i> , <i>K. pneumoniae</i> , <i>H. influenzae</i>
14. Bacterial meningitis	<i>Escherichia coli</i> , <i>H. influenzae</i> , <i>Neisseria meningitidis</i> , <i>Streptococcus pneumoniae</i>
15. Bacterial endocarditis (Chapter 25)	<i>Staphylococcus aureus</i> , <i>Streptococcus viridans</i>
16. Other staphylococcal infections*	<i>S. aureus</i> , <i>S. epidermidis</i> , <i>S. saprophyticus</i>
17. Streptococcal infections*	<i>S. pyogenes</i> , <i>S. faecalis</i> , <i>S. pneumoniae</i> , <i>S. viridans</i>
18. E. coli infections (Urinary tract infection)	<i>Escherichia coli</i>
19. Clostridial diseases*	
i) Gas gangrene	<i>C. perfringens</i>
ii) Tetanus	<i>C. tetani</i>
iii) Botulism	<i>C. botulinum</i>
iv) Clostridial food poisoning	<i>C. perfringens</i>
v) Necrotising enterocolitis	<i>C. perfringens</i>
20. Tuberculosis (page 117)	<i>Mycobacterium tuberculosis</i>
21. Leprosy (page 127)	<i>Mycobacterium leprae</i>
22. Syphilis (page 130)	<i>Treponema pallidum</i>
23. Actinomycosis (page 132)	<i>Actinomyces israelii</i>
24. Nocardiosis	<i>Nocardia asteroides</i>

*Diseases discussed in this chapter.

liver (causing foci of parenchymal necrosis), in the gall bladder (producing typhoid cholecystitis), and in the spleen (resulting in splenic reactive hyperplasia).

PATHOLOGIC CHANGES. The lesions are observed in the intestines as well as in other organs.

1. INTESTINAL LESIONS. *Macroscopically*, terminal ileum is affected most often, but lesions may be seen in the jejunum and colon. The Peyer's patches show oval typhoid ulcers with their *long axis along the length of the bowel*, (c.f. tuberculous ulcers of small intestine, which are transverse to long axis). The base of the ulcers is black due to sloughed mucosa. The margins of the ulcers are slightly raised due to inflammatory oedema and cellular proliferation. There is never significant fibrosis and hence fibrous stenosis seldom occurs in healed typhoid lesions. The

regional lymph nodes are invariably enlarged (Fig. 14.1,A).

Microscopically, there is hyperaemia, oedema and cellular proliferation consisting of phagocytic histiocytes (showing characteristic erythrophagocytosis), lymphocytes and plasma cells. Though enteric fever is an example of acute inflammation, neutrophils are invariably absent from the cellular infiltrate and this is reflected in the leucopenia with neutropenia and relative lymphocytosis in the peripheral blood (Fig. 14.1,B).

The main **complications** of the intestinal lesions of typhoid are perforation of the ulcers and haemorrhage.

2. OTHER LESIONS. Besides the intestinal involvement, various other organs and tissues showing pathological changes in enteric fever are as under:

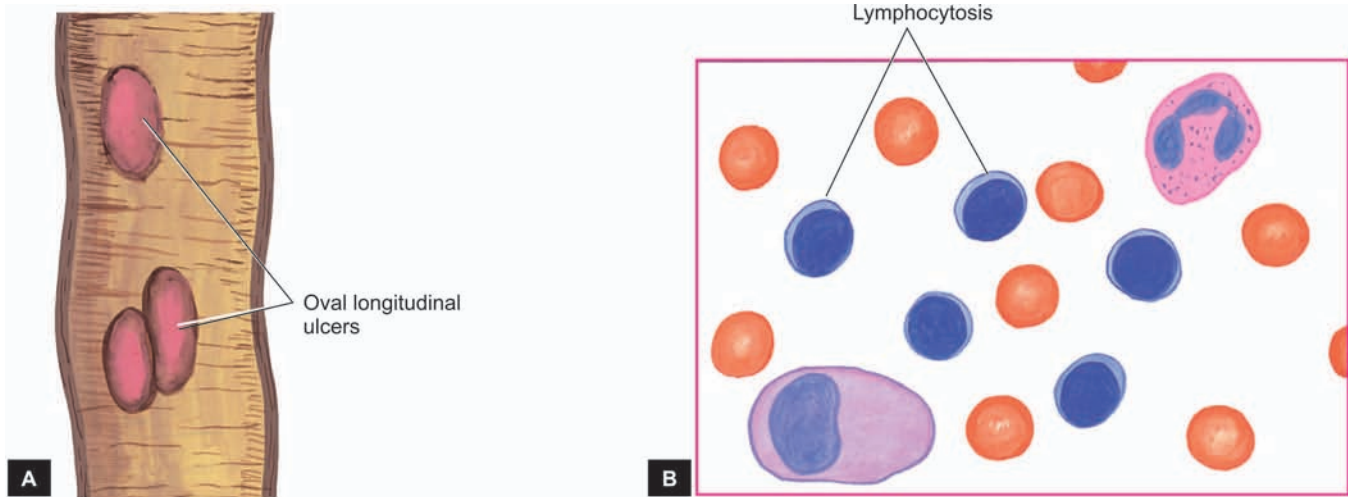


FIGURE 14.1

A, Typhoid ulcers in the small intestine appear characteristically oval with their long axis parallel to the long axis of the bowel. B, Blood picture in typhoid fever showing neutropenia and relative lymphocytosis.

- i) Mesenteric lymph nodes—haemorrhagic lymphadenitis.
- ii) Liver—foci of parenchymal necrosis.
- iii) Gallbladder—typhoid cholecystitis.
- iv) Spleen—splenomegaly with reactive hyperplasia.
- v) Kidneys—nephritis.
- vi) Abdominal muscles—Zenker's degeneration.
- vii) Joints—arthritis.
- viii) Bones—osteitis.
- ix) Meninges—Meningitis.
- x) Testis—Orchitis.

Persistence of organism in the gallbladder or urinary tract may result in passage of organisms in the faeces or urine creating a 'carrier state' which is a source of infection to others.

BACTERIAL FOOD POISONING

This is a form of acute bacterial illness that occurs following ingestion of food or water contaminated with bacteria other than those that cause specific acute intestinal infections like typhoid, paratyphoid, cholera or dysentery bacilli. The illness results from either bacterial invasion or bacterial toxigenic effect on the bowel.

The commonest causes of bacterial food poisoning resulting in enteritis or enterocolitis are as under:

1. Staphylococcal food poisoning. *Staphylococcus aureus* infection acquired from contaminated food produces either mild food poisoning by enterotoxins, or

may cause more severe form of the illness called *pseudomembranous enterocolitis* described below. Staphylococcal food poisoning occurs due to liberation of enterotoxins by the bacteria.

2. Clostridial food poisoning. Infection with anaerobic organisms *Clostridium welchii*, following consumption of contaminated meat results in acute food poisoning. The illness occurs both by bacterial invasion as well as by toxins.

3. Botulism. This is a severe form of paralysing illness caused by ingestion of organism, *Clostridium botulinum*, which produces neurotoxin.

4. Salmonella food poisoning (Salmonellosis). This is an infection (and not caused by toxins) occurring due to food contaminated by *S. typhimurium* or *S. enteritidis*. The condition manifests with fever, vomiting, and diarrhoea. Death may result from depletion of water and electrolytes.

PLAGUE

Plague is caused by *Yersinia (Pasteurella) pestis* which is a small gram-negative coccobacillus that grows rapidly on most culture media. Direct identification of the organism in tissues is possible by fluorescence antisera methods.

Plague has been a great killer since 14th century and is known to have wiped out populations of cities. However, the modern Europe is plague free, probably

due to widespread use of arsenic as rat poison. Currently, the world over, Vietnam and Tanzania have most cases of plague. However, an outbreak in Surat in the state of Gujarat in Western part of India in 1994 alarmed the world once again that we are not totally free of this dreaded 'black death'.

Plague is a zoonotic disease and spreads by rodents, primarily by rats, both wild and domestic; others being squirrels and rabbits.

Infection to humans occurs by rat-flea or by inhalation. After the organisms enter the blood stream, they reach the draining lymph nodes where, rather than being phagocytosed by phagocytic cells, they proliferate rapidly giving rise to tender lymphadenopathy. This occurs within 24-48 hours of infection and is accompanied by chills, fever, myalgia, nausea, vomiting and marked prostration. If untreated, death occurs from disseminated intravascular coagulation (DIC) within 1 to 2 days with development of widespread petechiae and ecchymoses leading to gangrene and hence the name *black death*. In other cases, death results from multi-organ failure due to profound toxæmia. The

patient and his fluids are highly infectious and can be transmitted by arthropods as well as person-to-person contact, giving rise to secondary cases.

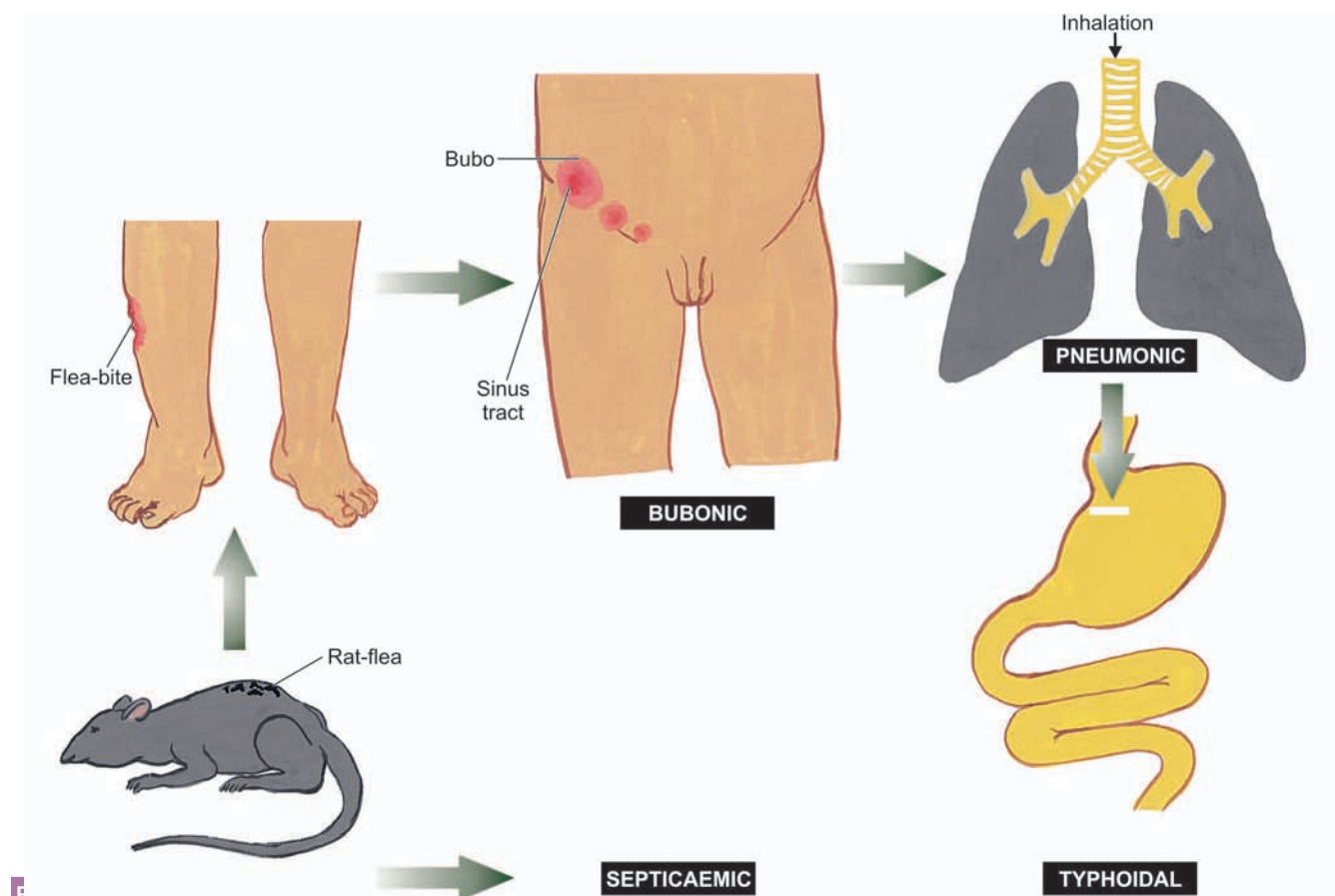
Virulence of the organism *Y. pestis* is attributed to the elaboration of plague toxins: pestin and lipopolysaccharide endotoxin.

PATHOLOGIC CHANGES. Following forms of plague are recognised (Fig. 14.2):

1. Bubonic plague, the most common
2. Pneumonic plague
3. Typhoidal plague
4. Septicaemic plague

BUBONIC PLAGUE. This form is characterised by rapid appearance of tender, fluctuant and enlarged regional lymph nodes, several centimeters in diameter, and may have discharging sinuses on the skin. *Microscopically*, the features are as under:

- Effaced architecture of lymph nodes due to necrosis in and around the affected nodes.
- Multiple necrotising granulomas.



Forms of plague.

- Characteristic mononuclear inflammatory response.
- Masses of proliferating bacilli in sinusoids of lymph nodes.
- Cellulitis in the vicinity.

PNEUMONIC PLAGUE. This is the most dreaded form of plague that occurs by inhalation of bacilli from air-borne particles of carcasses of animals or from affected patient's cough. It is characterised by occurrence of bronchopneumonia, with the following conspicuous microscopic features:

- Necrosis of alveolar walls.
- Intense hyperaemia and haemorrhages.
- Numerous bacilli in the alveolar lumina.
- Characteristic mononuclear inflammatory response with very scanty neutrophils.

TYPHOIDAL PLAGUE. This form of plague is unassociated with regional lymphadenopathy. The lesions in typhoidal plague are as follows:

- Necrotic foci in visceral lymphoid tissue.
- Necrotic areas in parenchymal visceral organs.
- G.I. manifestations with diarrhoea and pain abdomen.

SEPTICAEMIC PLAGUE. This is a form of progressive, fulminant bacterial infection associated with profound septicaemia in the absence of affarent regional lymphadenitis.

ANTHRAX

Anthrax is a bacterial disease of antiquity caused by *Bacillus anthracis* that spreads from animals to man. The disease is widely prevalent in cattle and sheep but human infection is rare. However, much of knowledge on human anthrax has been gained owing to fear of use of these bacteria for military purpose or for "bio-terrorism" (other microbial diseases in this list include: botulism, pneumonic plague, smallpox). Recently, the human form of disease has attracted a lot of attention of the media and the civilized world due to its use in the form of anthrax-laced letters sent by possible terrorist groups as a retaliatory biological weapon against the US interest subsequent to punitive attacks by the US on Afghanistan as an aftermath of September 11, 2001 terrorist attacks in the US. In India, anthrax in animals is endemic in South due to large unprotected and uncontrolled population of live-stock population. But anthrax is also prevalent in human beings in India; in Pondicherry alone 35 cases have been reported.

ETIOPATHOGENESIS. The causative organism, *Bacillus anthracis*, is a gram-positive, aerobic bacillus, 4.5 µm long. It is a spore-forming bacillus and the spores so formed outside the body are quite resistant. The disease occurs as an exogenous infection by contact with soil or animal products contaminated with spores.

Depending upon the portal of entry, three types of human anthrax is known to occur:

- i) **Cutaneous form** by direct contact with skin and is most common.
- ii) **Pulmonary form** by inhalation, also called as "wool-sorters' disease" and is most fatal.
- iii) **Gastrointestinal form** by ingestion and is rare.

The mechanism of infection includes spread of bacilli from the portal of entry to the regional lymph nodes through lymphatics where the bacteria proliferate. There is delayed accumulation of polymorphs and macrophages. Macrophages also play a role in expression of bacterial toxicity; bacterial toxin is quite lethal to macrophages.

PATHOLOGIC CHANGES. The characteristic lesions of anthrax are haemorrhage, oedema and necrosis at the portal of entry.

1. Cutaneous anthrax is the most common and occurs in two forms: one type is characterized by necrotic lesion due to vascular thrombosis, haemorrhage and acellular necrosis, while the other form begins as a pimple at the point of entry of *B. anthracis* into the abraded exposed skin, more often in the region of hands and the head and neck. The initial lesion develops into a vesicle or blister containing clear serous or blood-stained fluid swarming with anthrax bacilli which can be identified readily by smear examination. The bursting of the blister is followed by extensive oedema and black tissue necrosis resulting in formation of severe 'malignant pustule'. Regional lymph nodes are invariably involved alongwith profound septicaemia.

2. Pulmonary anthrax (wool-sorters' disease) occurring from inhalation of spores of *B. anthracis* in infectious aerosols results in rapid development of malignant pustule in the bronchus. This is followed by development of primary extensive necrotising pneumonia and haemorrhagic mediastinitis which is invariably fatal.

3. Intestinal anthrax is rare in human beings and is quite similar to that seen in cattle. Septicaemia and death often results in this type too. The lesions consist

of mucosal oedema, small necrotic ulcers, massive fluid loss and haemorrhagic mesenteric lymphadenitis.

Besides, anthrax septicaemia results in spread of infection to all other organs.

LABORATORY DIAGNOSIS. Anthrax can be diagnosed by a few simple techniques:

Smear examination: Gram stained smear shows rod-shaped, spore-forming, gram-positive bacilli. Endospores are detectable by presence of unstained defects or holes within the cell.

Culture: Anthrax bacteria grow on sheep blood agar as flat colonies with an irregular margin (medusa head).

Anthrax contaminated work surfaces, materials and equipment must be decontaminated with 5% hypochlorite or 5% phenol.

WHOOPING COUGH (PERTUSSIS)

Whooping cough is a highly communicable acute bacterial disease of childhood caused by *Bordetella pertussis*. The use of DPT vaccination has reduced the prevalence of whooping cough in different populations.

The causative organism, *B. pertussis*, has strong tropism for the brush border of the bronchial epithelium. The organisms proliferate here and stimulate the bronchial epithelium to produce abundant tenacious mucus. Within 7-10 days after exposure, catarrhal stage begins which is the most infectious stage. There is low grade fever, rhinorrhoea, conjunctivitis and excess tear production. Paroxysms of cough occur with characteristic 'whoop'. The condition is self-limiting but may cause death due to asphyxia in infants. *B. pertussis* produces a heat-labile toxin, a heat-stable endotoxin, and a lymphocytosis-producing factor called histamine-sensitising factor.

Microscopically, the lesions in the respiratory tract consist of necrotic bronchial epithelium covered by thick mucopurulent exudate. In severe cases, there is mucosal erosion and hyperaemia. The peripheral blood shows marked lymphocytosis upto 90% and enlargement of lymphoid follicles in the bronchial mucosa and peribronchial lymph nodes.

GRANULOMA INGUINALE

Granuloma inguinale is a sexually-transmitted disease affecting the genitalia and inguinal and perianal regions caused by *Calymmatobacterium donovani*. The disease is common in tropical and subtropical countries such as

New Guinea, Australia and India. The organism inhabits the intestinal tract. The infection is transmitted through vaginal or anal intercourse and by autoinoculation. The incubation period varies from 2 to 4 weeks. Initially, the lesion is in the form of a papule, a subcutaneous nodule or an ulcer. Within a few weeks, it develops into a raised, soft, painless, reddish ulcer with exuberant granulation tissue. Depending upon whether the individual is heterosexual or homosexual, the lesions are located on the penis, scrotum, genitocrural folds and inguinal folds, or in the perianal and anal area respectively. Regional lymphadenopathy generally does not occur.

Microscopically, the margin of the ulcer shows epithelial hyperplasia. The ulcer bed shows neutrophilic abscesses. The dermis and subcutaneous tissues are infiltrated by numerous histiocytes containing many bacteria called *Donovan bodies*, and lymphocytes, plasma cells and neutrophils. These organisms are best demonstrated by silver impregnation techniques.

STAPHYLOCOCCAL INFECTIONS

Staphylococci are gram-positive cocci which are present everywhere—in the skin, umbilicus, nasal vestibule, stool etc. Three species are pathogenic to human beings: *Staph. aureus*, *Staph. epidermidis* and *Staph. saprophyticus*. Most staphylococcal infections are caused by *Staph. aureus*. Staphylococcal infections are among the commonest antibiotic-resistant hospital-acquired infection in surgical wounds.

A wide variety of suppurative diseases are caused by *Staph. aureus* which includes the following (Fig. 14.3):

1. Infections of skin. Staphylococcal infections of skin are quite common. The infection begins from lodgement of cocci in the hair root due to poor hygiene and results in obstruction of sweat or sebaceous gland duct. This is termed *folliculitis*. Involvement of adjacent follicles results in larger lesions called *furuncle*. Further spread of infection horizontally under the skin and subcutaneous tissue causes *carbuncle* or *cellulitis*. *Styes* are staphylococcal infection of the sebaceous glands of Zeis, the glands of Moll and eyelash follicles. *Impetigo* is yet another staphylococcal skin infection common in school children in which there are multiple pustular lesions on face forming honey-yellow crusts. *Breast abscess* may occur following delivery when staphylococci are transmitted from infant having neonatal sepsis or due to stasis of milk.

2. Infections of burns and surgical wounds. These are quite common due to contamination from the

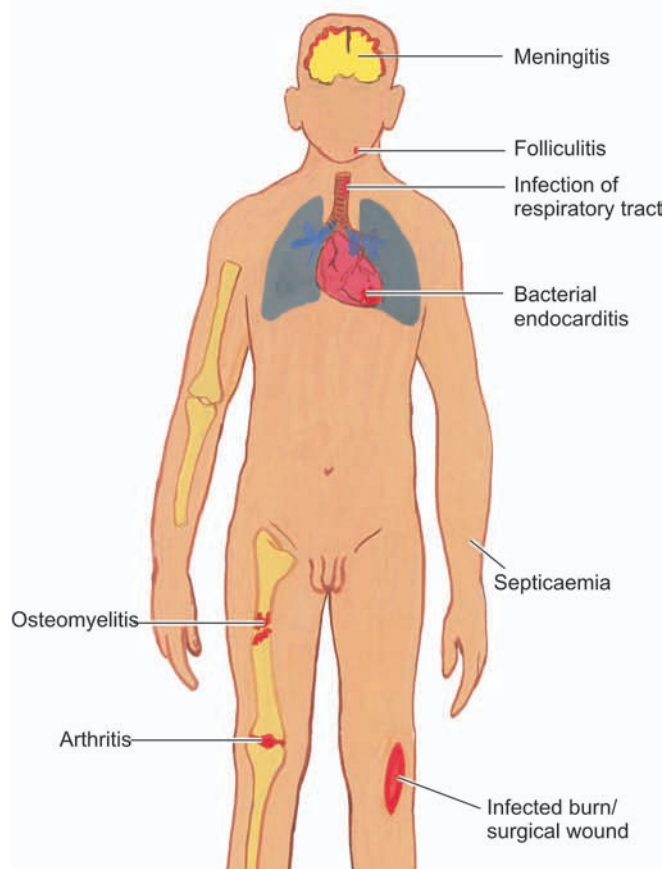


FIGURE 14.3

Suppurative diseases caused by *Staphylococcus aureus*.

patient's own nasal secretions or from hospital staff. Elderly, malnourished, obese and neonates have increased susceptibility.

3. Infections of the upper and lower respiratory tract. Small children under 2 years of age get staphylococcal infections of the respiratory tract commonly. These include pharyngitis, bronchopneumonia, staphylococcal pneumonia and its complications.

4. Bacterial arthritis. Septic arthritis in the elderly is caused by *Staph. aureus*.

5. Infection of bone (Osteomyelitis). Young boys having history of trauma or infection may develop acute staphylococcal osteomyelitis (page 416).

6. Bacterial endocarditis. Acute and subacute bacterial endocarditis are complications of infection with *Staph. aureus* and *Staph. epidermidis* (page 323).

7. Bacterial meningitis. Surgical procedures on central nervous system may lead to staphylococcal meningitis.

8. Septicaemia. Staphylococcal septicaemia may occur in patients with lowered resistance or in patients having underlying staphylococcal infections. Patients present with features of bacteraemia such as shaking chills and fever.

9. Toxic shock syndrome. Toxic shock syndrome is a serious complication of staphylococcal infection characterised by fever, hypotension and exfoliative skin rash. The condition affects young menstruating women who use tampons of some brands which when kept inside the vagina cause absorption of staphylococcal toxins from the vagina.

STREPTOCOCCAL INFECTIONS

Streptococci are also gram-positive cocci but unlike staphylococci, they are more known for their non-suppurative autoimmune complications than suppurative inflammatory responses. Streptococcal infections occur throughout the world but their problems are greater in underprivileged populations where antibiotics are not instituted readily.

The following groups and subtypes of streptococci have been identified and implicated in different streptococcal diseases (Fig. 14.4):

1. *Group A or Streptococcus pyogenes*, also called β -haemolytic streptococci, are involved in causing upper respiratory tract infection and cutaneous infections (erysipelas). In addition, beta haemolytic streptococci are involved in autoimmune reactions in the form of rheumatic heart disease (RHD).
2. *Group B or Streptococcus agalactiae* produces infections in the newborn and is involved in non-suppurative post-streptococcal complications such as RHD and acute glomerulonephritis.
3. *Group C and G streptococci* are responsible for respiratory infections.
4. *Group D or Streptococcus faecalis*, also called enterococci are important in causation of urinary tract infection, bacterial endocarditis, septicaemia etc.
5. *Untypable α -haemolytic streptococci* such as *Streptococcus viridans* constitute the normal flora of the mouth and may cause bacterial endocarditis.
6. *Pneumococci or Streptococcus pneumoniae* are etiologic agents for bacterial pneumonias, meningitis and septicaemia.

CLOSTRIDIAL DISEASES

Clostridia are gram-positive spore-forming anaerobic microorganisms found in the gastrointestinal tract of herbivorous animals and man. These organisms may

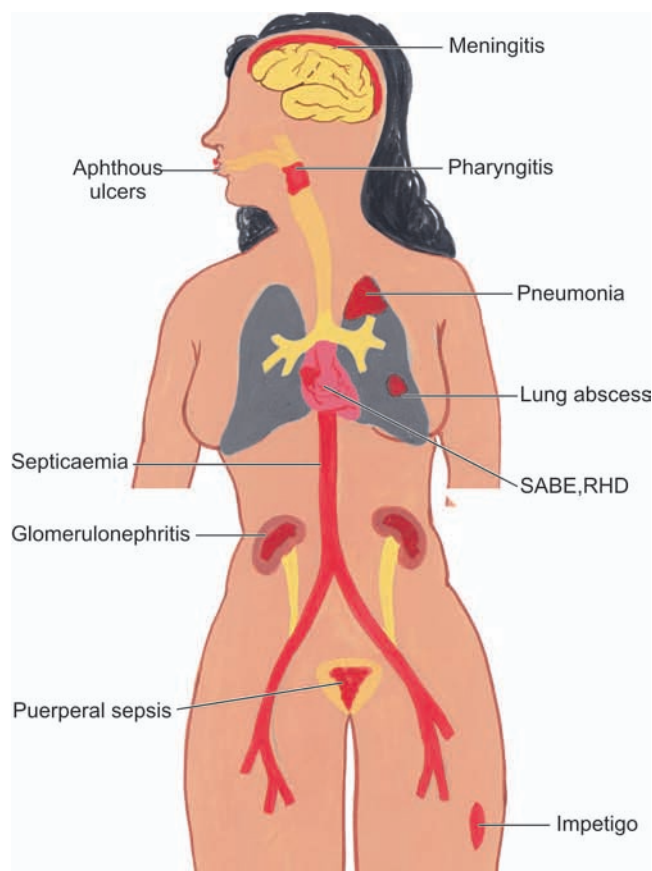


FIGURE 14.4

Diseases caused by streptococci.

undergo vegetative division under anaerobic conditions, and sporulation under aerobic conditions. These spores are passed in faeces and can survive in unfavourable conditions. On degeneration of these microorganisms, the plasmids are liberated which produce many toxins responsible for the following clostridial diseases depending upon the species (Fig. 14.5):

1. Gas gangrene by *C. perfringens*
2. Tetanus by *C. tetani*
3. Botulism by *C. botulinum*
4. Clostridial food poisoning by *C. perfringens*
5. Necrotising enterocolitis by *C. perfringens*.

GAS GANGRENE. Gas gangrene is a rapidly progressive and fatal illness in which there is myonecrosis of previously healthy skeletal muscle due to elaboration of myotoxins by some species of clostridia. In majority of cases (80-90%), the source of myotoxins is *C. perfringens* Type A; others are *C. novyi* and *C. septicum*. Generally, traumatic wounds and surgical procedures are followed by contamination with clostridia and

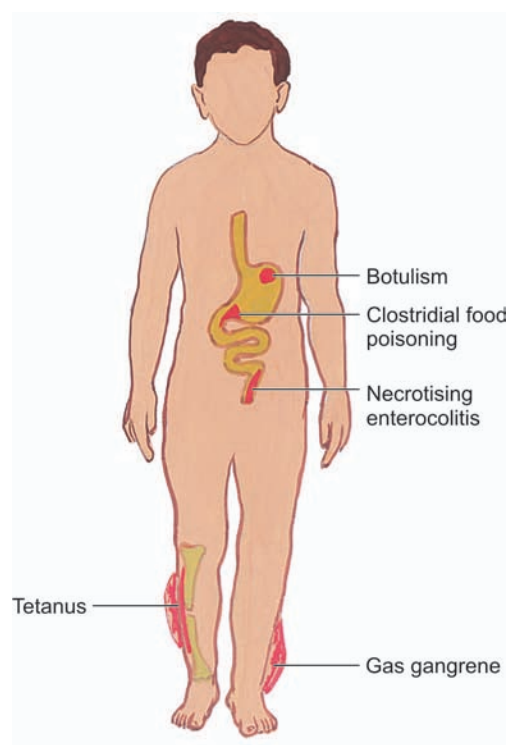


FIGURE 14.5

Diseases caused by clostridia.

become the site of myonecrosis (Fig. 14.5). The incubation period is 2 to 4 days. The most common myotoxin produced by *C. perfringens* Type A is the alpha toxin which is a lecithinase. The prevention of gas gangrene lies in debridement of damaged tissue in which the clostridia thrive. The lesion has serosanguineous discharge with odour and contains gas bubbles. There is very scanty inflammatory reaction at the site of gas gangrene.

TETANUS. Tetanus or 'lock jaw' is a severe acute neurologic syndrome caused by tetanus toxin, tetanospasmin, which is a neurotoxic exotoxin elaborated by *C. tetani*. The spores of the microorganism present in the soil enter the body through a penetrating wound. In underdeveloped countries, tetanus in neonates is seen due to application of soil or dung on the umbilical stump. The degenerated microorganisms liberate the tetanus neurotoxin which causes neuronal stimulation and spasm of muscles (Fig. 14.5). The incubation period of the disease is 1-3 weeks. The earliest manifestation is lock-jaw or trismus. Rigidity of muscles of the back causes backward arching or opisthotonos. Death occurs due to spasm of respiratory and laryngeal muscles.

BOTULISM. Botulism is characterised by symmetric paralysis of cranial nerves, limbs and trunk. The condition occurs following ingestion of food contaminated with neurotoxins of *C. botulinum* and less often by contamination of a penetrating wound. The spores of *C. botulinum* are capable of surviving in unfavourable conditions and contaminate vegetables and other foods, especially if improperly stored or canned. The symptoms of botulism begin to appear within 12 to 36 hours of ingestion of food containing the neurotoxins (type A to type G). The toxins resist gastric digestion and are absorbed from the upper portion of small intestine and enter the blood. On reaching the cholinergic nerve endings, the toxin binds to membrane receptors and inhibits release of acetylcholine resulting in paralysis and respiratory failure (Fig. 14.5).

CLOSTRIDIAL FOOD POISONING. Clostridial food poisoning is caused by enterotoxin elaborated by *C. perfringens*. Out of five serotypes of *C. perfringens*, type A and C produce alpha-enterotoxin that causes food poisoning (Fig. 14.5). These serotypes of organism are omnipresent in the environment and thus clostridial poisoning occurs throughout the world. Food poisoning from *C. perfringens* is mostly from ingestion of meat and its products which have been allowed to dry resulting in dehydration and anaerobic conditions suitable for growth of *C. perfringens*. The contaminated meat contains vegetative form of the organism and no preformed enterotoxin (unlike botulism where preformed neurotoxin of *C. botulinum* is ingested). On ingestion of the contaminated meat, alpha-enterotoxin is produced in the intestine. Symptoms of the food poisoning appear within 12 hours of ingestion of contaminated meat and recovery occurs within 2 days.

NECROTISING ENTEROCOLITIS. Necrotising enterocolitis or 'pig bel' is caused by beta-enterotoxin produced by *C. perfringens* Type C. The condition occurs especially in undernourished children who suddenly indulge in overeating such as was first reported participation in pig feasts by poor children in New Guinea and hence the name 'pig bel'. Adults do not develop the condition due to good antibody response.

Ingestion of contaminated pork by malnourished children who normally take protein-deficient vegetarian diet causes elaboration of beta-enterotoxin. The symptoms appear within 48 hours after ingestion of contaminated meat. These include: severe abdominal pain, distension, vomiting and passage of bloody stools. Milder form of disease runs a course similar to other forms of gastroenteritis while fulminant 'pig bel' may result in death of the child.

Grossly, the disease affects small intestine segmentally. The affected segment of bowel shows green, necrotic pseudomembrane covering the necrotic mucosa and there is associated peritonitis. Advanced cases may show perforation of the bowel wall.

Microscopically, there is transmural infiltration by acute inflammatory cell infiltrate with changes of mucosal infarction, oedema and haemorrhage. The pseudomembrane consists of necrotic epithelium with entangled bacilli.

DISEASES CAUSED BY FUNGI

Of the large number of known fungi, only a few are infective to human beings. Many of the human fungal infections are opportunistic i.e. they occur in conditions with impaired host immune mechanisms. Such conditions include defective neutrophil function, administration of corticosteroids, immunosuppressive therapy and immunodeficiency states (congenital and acquired). A list of common fungal infections of human beings is given in Table 14.3. A few important representative examples are discussed below.

MYCETOMA

Mycetoma is a chronic suppurative infection involving a limb, shoulder or other tissues and is characterised by draining sinuses. The material discharged from the sinuses is in the form of grains consisting of colonies of fungi or bacteria. Mycetomas are of 2 main types:

- *Mycetomas* caused by actinomyces (higher bacteria) comprising about 60% of cases; and
- *Eumycetomas* caused by true fungi comprising the remaining 40% of the cases.

TABLE 14.3: Diseases Caused by Fungi.

DISEASE	ETIOLOGIC AGENT
1. <i>Mycetoma</i> *	<i>Madurella mycetomatis</i>
2. <i>Aspergillosis</i>	<i>Aspergillus fumigatus</i> , <i>A. flavus</i> , <i>A. niger</i>
3. <i>Blastomycosis</i>	<i>Blastomyces dermatitidis</i>
4. <i>Candidiasis</i> *	<i>Candida albicans</i>
5. <i>Coccidioidomycosis</i>	<i>Coccidioides immitis</i>
6. <i>Cryptococcosis</i>	<i>Cryptococcus neoformans</i>
7. <i>Histoplasmosis</i>	<i>Histoplasma capsulatum</i>
8. <i>Rhinosporidiosis</i>	<i>Rhinosporidium seeberi</i>
9. <i>Superficial mycosis</i> *	<i>Microsporum</i> , <i>Trichophyton</i> , <i>Epidermophyton</i>

*Conditions discussed in this chapter.

Most common fungi causative for eumycetoma are *Madurella mycetomatis* or *Madurella grisea*, both causing black granules from discharging sinuses. Eumycetomas are particularly common in Northern and tropical Africa, Southern Asia and tropical America. The organisms are inoculated directly from soil into bare feet, from carrying of contaminated sacks on the shoulders, and into the hands from infected vegetation.

PATHOLOGIC CHANGES. After several months of infection, the affected site, most commonly foot, is swollen and hence the name 'madura foot'. The lesions extend deeply into the subcutaneous tissues, along the fascia and eventually invade the bones. They drain through sinus tracts which discharge purulent material and grains. The surrounding tissue shows granulomatous reaction.

CANDIDIASIS

Candidiasis is an opportunistic fungal infection caused most commonly by *Candida albicans* and occasionally by *Candida tropicalis*. In human beings, *Candida* species are present as normal flora of the skin and mucocutaneous areas, intestines and vagina. The organism becomes pathogenic when the balance between the host and the organism is disturbed. Various predisposing factors are: impaired immunity, prolonged use of oral contraceptives, long-term antibiotic therapy, corticosteroid therapy, diabetes mellitus, obesity, pregnancy etc.

PATHOLOGIC CHANGES. *Candida* produces superficial infections of the skin and mucous membranes, or may invade deeper tissues as described under:

1. **Oral thrush.** This is the commonest form of mucocutaneous candidiasis seen especially in early life. Full-fledged lesions consist of creamy white pseudomembrane composed of fungi covering the tongue, soft palate, and buccal mucosa. In severe cases, ulceration may be seen.
2. **Candidal vaginitis.** Vaginal candidiasis or monilial vaginitis is characterised clinically by thick, yellow, curdy discharge. The lesions form pseudomembrane of fungi on the vaginal mucosa. They are quite pruritic and may extend to involve the vulva (vulvovaginitis) and the perineum.
3. **Cutaneous candidiasis.** Candidal involvement of nail folds producing change in the shape of nail plate (paronychia) and colonisation in the intertriginous areas of the skin, axilla, groin, infra- and intermammary, intergluteal folds and interdigital spaces

are some of the common forms of cutaneous lesions caused by *Candida albicans*.

4. **Systemic candidiasis.** Invasive candidiasis is rare and is usually a terminal event of an underlying disorder associated with impaired immune system. The organisms gain entry into the body through an ulcerative lesion on the skin and mucosa or may be introduced by iatrogenic means such as via intravenous infusion, peritoneal dialysis or urinary catheterisation. The lesions of systemic candidiasis are most commonly encountered in kidneys as ascending pyelonephritis and in heart as candidal endocarditis.

SUPERFICIAL MYCOSIS

Dermatophytes are the most important example of cutaneous mycosis caused by *Microsporum*, *Trichophyton* and *Epidermophyton*. These superficial fungi are spread by direct contact or by fomites and infect tissues such as the skin, hair and nails. Examples of diseases pertaining to these tissues are as under:

- *Tinea capitis* characterised by patchy alopecia affecting the scalp and eyebrows.
- *Tinea barbae* is acute folliculitis of the beard.
- *Tinea corporis* is dermatitis with formation of erythematous papules.

The diagnosis of dermatophytosis is made by light microscopic examination of skin scrapings after addition of sodium or potassium hydroxide solution. Other methods include fungal culture and demonstration of fungus in tissue sections.

ASPERGILLOSIS

Aspergillosis is the most common fungal infection of the lung caused by *Aspergillus fumigatus*. The fungus exists as thin septate hyphae with dichotomous branching and grows best in cool, wet climate. The infection may result in *allergic bronchopulmonary aspergillosis*, *aspergilloma* and *necrotising bronchitis*. Immunocompromised persons develop more serious manifestations of aspergillus infection, especially in leukaemic patients on cytotoxic drug therapy. Extensive haematogenous spread of aspergillus infection may result in widespread changes in lung tissue due to arterial occlusion, thrombosis and infarction (COLOUR PLATE V: CL 17).

RHINOSPORIDIOSIS

Rhinosporidiosis is caused by a fungus, *Rhinosporidium seeberi*. Typically it occurs in a nasal polyp but may be found in other locations like nasopharynx, larynx and

conjunctiva. The disease is common in India and Sri Lanka.

Microscopically, besides the structure of inflammatory or allergic polyp, large number of organisms of the size of erythrocytes with chitinous wall are seen in the thick-walled *sporangia*. Each sporangium may contain a few thousand spores. On rupture of a sporangium, the spores are discharged into the submucosa or on to the surface of the mucosa. The intervening tissue consists of inflammatory granulation tissue (plasma cells, lymphocytes, histiocytes, neutrophils) while the overlying epithelium shows hyperplasia, focal thinning and occasional ulceration (Fig. 14.6).

DISEASES CAUSED BY VIRUSES

Viral diseases are the most common cause of human illness. However, many of the viral infections remain asymptomatic while others produce viral disease. Another peculiar feature of viral infection is that a single etiologic agent may produce different diseases in the same host depending upon host immune response and age at infection e.g. varicella-zoster virus is causative for chickenpox as well as herpes zoster. Viruses are essentially intracellular parasites. Depending upon their nucleic acid genomic composition, they may be single-stranded or double-stranded, RNA or DNA viruses. A

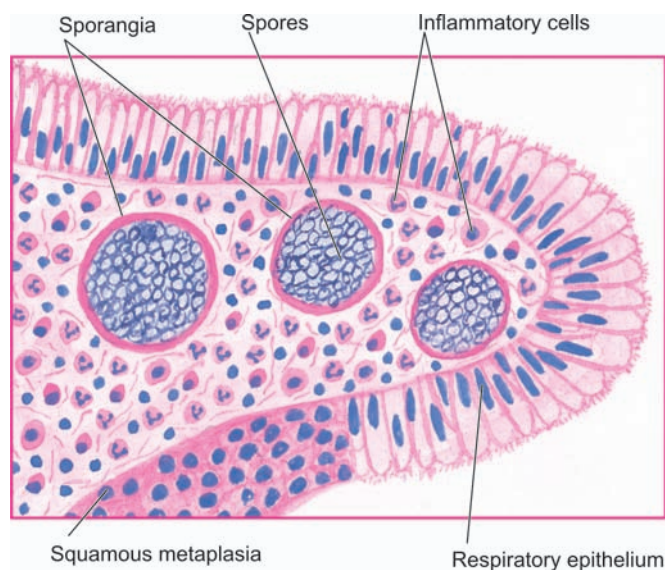


FIGURE 14.6

Rhinosporidiosis in a nasal polyp. The spores are present in sporangia as well as are intermingled in the inflammatory cell infiltrate.

list of common viruses and diseases caused by them is given in Table 14.4. Oncogenic viruses and their role in neoplasms are discussed in Chapter 21. A few common and important viral diseases are described below.

VIRAL HAEMORRHAGIC FEVERS

Viral haemorrhagic fevers are a group of acute viral infections which have common features of causing haemorrhages, shock and sometimes death. Viruses causing haemorrhagic fevers were earlier called *arthropod-borne (or arbo)* viruses since their transmission was considered to be from arthropods to humans. However, now it is known that all such viruses are not transmitted by arthropod vectors alone and hence now such haemorrhagic fevers are classified according to the routes of transmission and other epidemiologic features into 4 groups:

- Mosquito-borne (e.g. yellow fever, dengue fever, Rift Valley fever)
- Tick-borne (e.g. Crimean haemorrhagic fever, Kyasanur Forest disease)
- Zoonotic (e.g. Korean haemorrhagic fever, Lassa fever)
- Marburg virus disease and Ebola virus disease by unknown route.

Of these, mosquito-borne viral haemorrhagic fevers in which *Aedes aegypti* mosquitoes are vectors are the most common problem the world over, especially in underdeveloped countries. Two important examples of *Aedes* mosquito-borne viral haemorrhagic fevers are yellow fever and dengue fever, which are discussed below.

Yellow Fever

Yellow fever is the oldest known viral haemorrhagic fever restricted to some regions of Africa and South America. Monkeys carry the virus without suffering from illness and the virus is transmitted from them to humans by *Aedes aegypti* as vector.

Yellow fever is characterised by the following clinical features: Sudden onset of high fever, chills, myalgia, headache, jaundice, hepatic failure, renal failure, bleeding disorders and hypotension.

PATHOLOGIC CHANGES. Major pathologic changes are seen in the liver and kidneys.

Liver. The characteristic changes include:

- i) midzonal necrosis;
- ii) Councilman bodies; and
- iii) microvesicular fat.

TABLE 14.4: Diseases Caused by Viruses.

DISEASE	ETIOLOGIC AGENT
1. <i>Viral haemorrhagic fevers*</i>	Arthropod-borne (arbo) viruses
2. <i>Severe acute respiratory syndrome (SARS, Bird flu)*</i>	Influenza virus type A
3. <i>Viral encephalitis</i>	Arthropod-borne (arbo) viruses
4. <i>Rabies*</i>	Rabies virus (arboviruses)
5. <i>Poliomyelitis</i>	Poliovirus
6. <i>Smallpox (Variola)</i>	Variola virus
7. <i>Chickenpox (varicella)*</i>	Varicella-zoster virus
8. <i>Herpes simplex and herpes genitalis*</i>	Herpes simplex virus (HSV-I) and (HSV-II)
9. <i>Herpes zoster*</i>	Varicella-zoster virus
10. <i>Lymphogranuloma venereum*</i>	<i>Chlamydia trachomatis</i>
11. <i>Cat-scratch disease*</i>	<i>Bartonella henselae</i>
12. <i>Viral hepatitis (page 361)</i>	Hepatotropic viruses
13. <i>Cytomegalovirus inclusion disease</i>	Cytomegalovirus (CMV)
14. <i>Infectious mononucleosis (page 487)</i>	Epstein-Barr virus (EBV)
15. <i>Measles (Rubeola)</i>	Measles virus
16. <i>German measles (Rubella)</i>	Rubella virus
17. <i>Mumps (page 351)</i>	Mumps virus
18. <i>Viral respiratory infections</i>	Adenovirus, echovirus, rhinovirus, coxsackie virus, influenza A,B and C etc.
19. <i>Viral gastroenteritis</i>	Rotaviruses, Norwalk-like viruses

*Diseases discussed in this chapter.

Kidneys. The kidneys show the following changes:

- coagulative necrosis of proximal tubules;
- accumulation of fat in the tubular epithelium; and
- haemorrhages.

Patients tend to recover without sequelae and death rate is less than 5%, death resulting from hepatic or renal failure, and petechial haemorrhages in the brain.

Dengue Haemorrhagic Fever (DHF)

The word dengue is derived from African word 'denga' meaning fever with haemorrhages. Dengue is caused by virus transmitted by bites of mosquito *Aedes aegypti*; the transmission being highest during and after rainy season when mosquitos are numerous. DHF was first described in 1953 when it struck Philippines. An outbreak of DHF occurred in Delhi and neighbouring cities in 1996 claiming several lives. In 1997 too, some cases of DHF have been reported in post-monsoon period in Delhi.

Dengue occurs in two forms:

- Dengue fever or break-bone fever* in an uncomplicated way is a self-limited febrile illness affecting muscles and joints with severe back pain due to myalgia (and hence the name 'break-bone' fever).

- Dengue haemorrhagic fever (DHF)*, on the other hand, is a severe and potentially fatal form of acute febrile illness characterised by cutaneous and intestinal haemorrhages due to thrombocytopenia, haemoconcentration, hypovolaemic shock and neurologic disturbances. DHF is most common in children under 15 years of age.

Dengue virus infects blood monocytes, lymphocytes and endothelial cells. This initiates complement activation and consumptive coagulopathy including thrombocytopenia. The entire process takes place rapidly and may evolve over a period of a few hours. If patient is treated appropriately at this stage, there is rapid and dramatic recovery. But in untreated cases, *dengue shock syndrome* develops and death occurs.

PATHOLOGIC CHANGES. The predominant *organ changes in DHF* are due to:

- focal haemorrhages and congestion;
- increased vascular permeability resulting in oedema in different organs;
- coagulopathy with thrombocytopenia; and
- haemoconcentration.

The main derangement in **laboratory investigations in DHF** are:

- Leucopenia with relative lymphocytosis, sometimes with atypical lymphocytes

- ii) Thrombocytopenia
- iii) Elevated haematocrit due to haemoconcentration
- iv) X-ray chest showing bilateral pleural effusion
- v) Deranged liver function tests (elevated transaminases, hypoalbuminaemia and reversed A:G ratio)
- vi) Prolonged coagulation tests (prothrombin time, activated partial thromboplastin time and thrombin time)

Diagnosis of DHF is confirmed by:

- serologic testing for detection of antibodies;
- detection of virus by immunofluorescence method and monoclonal antibodies; and
- rapid methods such as reverse transcriptase-PCR and fluorogenic-ELISA.

At autopsy, the predominant organ changes observed are as follows:

- i) *Brain*: intracranial haemorrhages, cerebral oedema, dengue encephalitis.
- ii) *Liver*: enlarged; necrosis of hepatocytes and Kupffer cells, Reye's syndrome in children.
- iii) *Kidneys*: petechial haemorrhages and features of renal failure.
- iv) *Muscles and joints*: perivascular mononuclear cell infiltrate.

SEVERE ACUTE RESPIRATORY SYNDROME (SARS)

Severe acute respiratory syndrome (SARS) is the human form of bird flu or avian influenza having similar symptomatology. Recently, since the beginning of the year 2004, there have been outbreaks in poultry birds resulting in slaughtering of millions of infected chickens. There have been confirmed reports of spread of infection to human beings during this period in South-East Asia (particularly in Hong Kong, Vietnam, Thailand, South Korea, Japan, Indonesia, Taiwan and China) and its rapidly downhill and fatal clinical course; this has sent alarm bells all over world for quarantine.

ETIOPATHOGENESIS. Bird flu is caused by influenza viruses type A and primarily infects the birds, pigs and horses. Though it is not fatal for wild birds, it can kill poultry birds and people. Humans acquire infection through contaminated nasal, respiratory and faecal material from infected birds. An individual who has human flu and also gets infected with bird flu, then the hybrid virus so produced is highly contagious and causes lethal disease. The subtype of the virus in the current outbreak is H5N1. No person-to-person transmission has been reported. Humans do not have immune protection against avian viruses.

CLINICOPATHOLOGICAL FEATURES. Typically, the disease begins with influenza-like features such as fever, cough, sore throat, muscle aches and eye infection. Soon, the patient develops viral pneumonia and acute respiratory distress (hence the term SARS), and terminally kidney failure.

There is apprehension of an epidemic of SARS if the avian virus mutates and gains the ability to cause person-to-person infection. Since currently vaccine is yet being developed, the available measures are directed at prevention of infection and isolation of infected case.

VARICELLA ZOSTER VIRUS INFECTION

Varicella zoster virus is a member of herpes virus family and causes chickenpox (varicella) in non-immune individuals and herpes zoster (shingles) in those who had chickenpox in the past.

Varicella or chickenpox is an acute vesicular exanthem occurring in non-immune persons, especially children. The condition begins as an infection of the nasopharynx. On entering the blood stream, viraemia is accompanied by onset of fever, malaise and anorexia. Maculopapular skin rash, usually on upper trunk and face, develops in a day or two. This is followed by formation of vesicles which rupture and heal with formation of scabs. A few cases may develop complications which include pneumonia, hepatitis, encephalitis, carditis, orchitis, arthritis, and haemorrhages.

Herpes zoster or shingles is a recurrent, painful, vesicular eruption caused by reactivation of dormant varicella zoster virus in an individual who had chickenpox in the earlier years. The condition is infectious and spreads to children. The virus during the latent period resides in the dorsal root spinal ganglia or in the cranial nerve ganglia. On reactivation, the virus spreads from the ganglia to the sensory nerves and to peripheral nerves. Unlike chickenpox, the vesicles in shingles are seen in one or more of the sensory dermatomes and along the peripheral nerves. The lesions are particularly painful as compared with painless eruptions in chickenpox.

HERPES SIMPLEX VIRUS INFECTION

Two of the herpes simplex viruses (HSV)—type 1 and 2, cause 'fever blisters' and herpes genitalis respectively.

HSV-1 causes vesicular lesions on the skin, lips and mucous membranes. The infection spreads by close contact. The condition is particularly severe in

immunodeficient patients and neonates while milder attacks of infection cause fever blisters on lips, oral mucosa and skin. Severe cases may develop complications such as meningoencephalitis and keratoconjunctivitis. Various stimuli such as fever, stress and respiratory infection reactivate latent virus lying in the ganglia and result in recurrent attacks of blisters.

HSV-2 causes herpes genitalis characterised by vesicular and necrotising lesions on the cervix, vagina and vulva. Like HSV-1 infection, lesions caused by HSV-2 are also recurrent and develop in non-immune individuals. Latency of HSV-2 infection is similar to HSV-1 and the organisms are reactivated by stimuli such as menstruation and sexual intercourse.

LYMPHOGRANULOMA VENEREUM

Lymphogranuloma venereum (LGV) is a sexually-transmitted disease caused by *Chlamydia trachomatis* and is characterised by mucocutaneous lesions and regional lymphadenopathy. Though described here under viral infections, chlamydia are no more considered as filterable viruses as was previously thought but are instead intracellular gram-negative bacteria. LGV is worldwide in distribution but its prevalence rate is high in tropics and subtropics in Africa, South-East Asia and India.

The condition begins as a painless, herpes-like lesion on the cervix, vagina, or penis. The organisms are carried via lymphatics to regional lymph nodes. The involved lymph nodes are tender, fluctuant and may ulcerate and drain pus.

Microscopically, the lymph nodes have characteristic stellate-shaped abscesses surrounded by a zone of epithelioid cells (granuloma). Healing stage of the acute lesion takes place by fibrosis and permanent destruction of lymphoid structure.

CAT-SCRATCH DISEASE

Another condition related to LGV, cat-scratch disease, is caused by *Bartonella henselae*, an organism linked to rickettsiae but unlike rickettsiae this organism can be grown in culture. The condition occurs more commonly in children (under 18 years of age). There is regional nodal enlargement which appears about 2 weeks after cat-scratch, and sometimes after thorn injury. The lymphadenopathy is self-limited and regresses in 2-4 months.

Microscopically the changes in lymph node are characteristics:

- i) Initially, there is formation of non-caseating sarcoid-like granulomas.
- ii) Subsequently, there are neutrophilic abscesses surrounded by palisaded histiocytes and fibroblasts, an appearance simulating LGV discussed above.
- iii) The organism is extracellular and can be identified by silver stains.

RABIES

Rabies is a fatal form of encephalitis in humans caused by rabies virus. The virus is transmitted into the human body by a bite by infected carnivores e.g. dog, wolf, fox and bats. The virus spreads from the contaminated saliva of these animals. The organism enters a peripheral nerve and then travels to the spinal cord and brain. A latent period of 10 days to 3 months may elapse between the bite and onset of symptoms. Since the virus localises at the brainstem, it produces classical symptoms of difficulty in swallowing and painful spasm of the throat termed hydrophobia. Other clinical features such as irritability, seizure and delirium point towards viral encephalopathy. Death occurs within a period of a few weeks.

Microscopic examination shows characteristic Negri bodies which are intracytoplasmic, deeply eosinophilic inclusions present in neurons of the brainstem.

DISEASES CAUSED BY PARASITES

Diseases caused by parasites (protozoa and helminths) are quite common and comprise a very large group of infestations and infections in human beings. Parasites may cause disease due to their presence in the lumen of the intestine, due to infiltration into the blood stream, or due to their presence inside the cells. A short list of parasitic diseases is given in Table 14.5. These diseases form a distinct subject of study called Parasitology; only a few conditions are briefly considered below.

AMOEBIASIS

Amoebiasis is caused by *Entamoeba histolytica*, named for its lytic action on tissues. It is the most important intestinal infection of man. The condition is particularly more common in tropical and subtropical areas with poor sanitation.

The parasite occurs in 2 forms: a *trophozoite form* which is active adult form seen in the tissues and diarrhoeal stools, and a *cystic form* seen in formed stools but not in the tissues. The trophozoite form can be stained positively with PAS procedure in tissue sections while

TABLE 14.5: Diseases Caused by Parasites.

DISEASE	ETIOLOGIC AGENT
A. PROTOZOAL DISEASES	
1. <i>Chagas' disease (Trypanosomiasis)</i>	<i>Trypanosoma cruzi</i>
2. <i>Leishmaniasis (Kala-azar)</i>	<i>L. tropica</i> , <i>L. braziliensis</i> , <i>L. donovani</i>
3. <i>Malaria*</i>	<i>Plasmodium vivax</i> , <i>P. falciparum</i> , <i>P. ovale</i> , <i>P. malariae</i>
4. <i>Toxoplasmosis</i>	<i>Toxoplasma gondii</i>
5. <i>Pneumocystosis</i>	<i>Pneumocystis carinii</i>
6. <i>Amoebiasis*</i>	<i>Entamoeba histolytica</i>
7. <i>Giardiasis</i>	<i>Giardia lamblia</i>
B. HELMINTHIC DISEASES	
1. <i>Ascariasis</i>	<i>Ascaris lumbricoides</i>
2. <i>Enterobiasis (oxyuriasis)</i>	<i>Enterobius vermicularis</i>
3. <i>Hookworm disease</i>	<i>Ancylostoma duodenale</i>
4. <i>Trichinosis</i>	<i>Trichinella spiralis</i>
5. <i>Filariasis*</i>	<i>Wuchereria bancrofti</i>
6. <i>Visceral larva migrans</i>	<i>Toxocara canis</i>
7. <i>Cutaneous larva migrans</i>	<i>Strongyloides stercoralis</i>
8. <i>Schistosomiasis (Bilharziasis)</i>	<i>Schistosoma haematobium</i>
9. <i>Clonorchiasis</i>	<i>Clonorchis sinensis</i>
10. <i>Fascioliasis</i>	<i>Fasciola hepatica</i>
11. <i>Echinococcosis (Hydatid disease)*</i>	<i>Echinococcus granulosus</i>
12. <i>Cysticercosis*</i>	<i>Taenia solium</i>

*Diseases discussed in this chapter

amoebic cysts having four nuclei can be identified in stools. The cysts are the infective stage of the parasite and are found in contaminated water or food. The trophozoites are formed from the cyst stage in the intestine and colonise in the caecum and large bowel. The trophozoites as well as cysts are passed in stools but the trophozoites fail to survive outside or are destroyed by gastric secretions.

PATHOLOGIC CHANGES. The lesions of amoebiasis include amoebic colitis, amoeboma, amoebic liver abscess and spread to other sites (Fig. 14.7).

■ **Amoebic colitis**, the most common type of amoebic infection begins as a small area of necrosis of mucosa which may ulcerate. These ulcerative lesions may enlarge, develop undermining of margins of the ulcer due to lytic action of the trophozoite and have necrotic bed. Such chronic amoebic ulcers are described as flask-shaped ulcers due to their shape. The margin of the ulcer shows inflammatory response consisting of admixture of polymorphonuclear as well as mononuclear cells.

■ **Amoeboma** is the inflammatory thickening of the wall of large bowel resembling carcinoma of the

colon. *Microscopically*, the lesion consists of inflammatory granulation tissue, fibrosis and clusters of trophozoites at the margin of necrotic with viable tissue.

■ **Amoebic liver abscess** may be formed by invasion of the radicle of the portal vein by trophozoites. Amoebic liver abscess may be single or multiple. The amoebic abscess contains yellowish-grey amorphous liquid material in which trophozoites are identified at the junction of the viable and necrotic tissue.

■ **Other sites** where spread of amoebic infection may occur are peritonitis by perforation of amoebic ulcer of colon, extension to the lungs and pleura by rupture of amoebic liver abscess, haematogenous spread to cause amoebic carditis and cerebral lesions, cutaneous amoebiasis via spread of rectal amoebiasis or from anal intercourse.

MALARIA

Malaria is a protozoal disease caused by any one or combination of four species of plasmodia: *Plasmodium vivax*, *Plasmodium falciparum*, *Plasmodium ovale* and

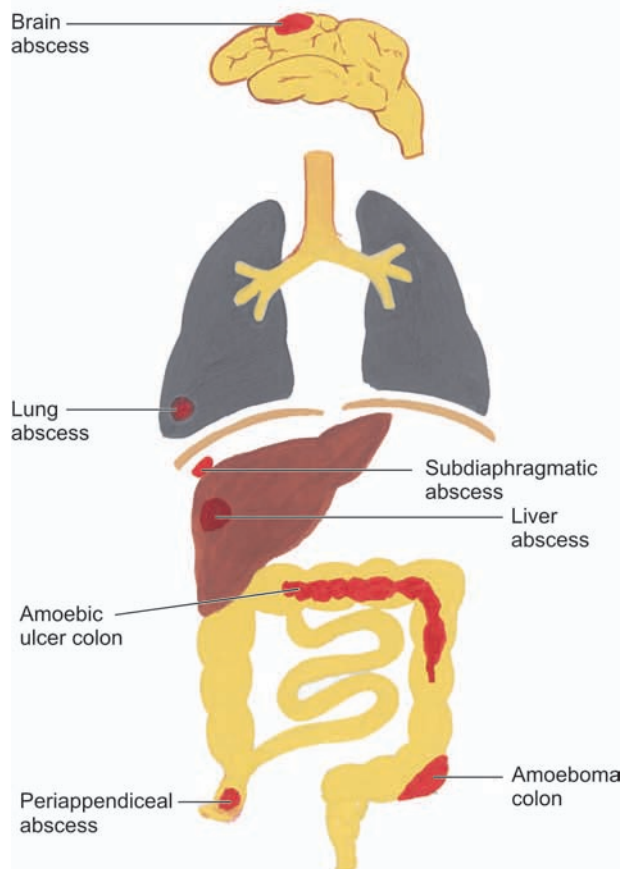


FIGURE 14.7

Complications of amoebiasis.

Plasmodium malariae. While *Plasmodium falciparum* causes malignant malaria, the other three species produce benign form of illness. These parasites are transmitted by bite of female *Anopheles* mosquito. The disease is endemic in several parts of the world, especially in tropical Africa, parts of South and Central America, India and South-East Asia.

The life cycle of plasmodia is complex and is diagrammatically depicted in Fig. 14.8,A. *P. falciparum* differs from other forms of plasmodial species in 4 respects:

- i) It does not have exo-erythrocytic stage.
- ii) Erythrocytes of any age are parasitised while other plasmodia parasitise juvenile red cells.
- iii) One red cell may contain more than one parasite.
- iv) The parasitised red cells are sticky causing obstruction of small blood vessels by thrombi, a feature which is responsible for extraordinary virulence of *P. falciparum*.

The main clinical features of malaria are cyclic peaks of high fever accompanied by chills, anaemia and splenomegaly.

PATHOLOGIC CHANGES. Parasitisation and destruction of erythrocytes are responsible for major pathologic changes as under (Fig. 14.8,B):

1. Malarial pigment liberated by destroyed red cells accumulates in the phagocytic cells of the reticuloendothelial system resulting in enlargement of the spleen and liver (*hepatosplenomegaly*).
2. In falciparum malaria, there is massive absorption of haemoglobin by the renal tubules producing *blackwater fever* (*haemoglobinuric nephrosis*).
3. At autopsy, *cerebral malaria* is characterised by congestion and petechiae on the white matter.
4. Parasitised erythrocytes in falciparum malaria are sticky and get attached to endothelial cells resulting in obstruction of capillaries of deep organs such as of the brain leading to hypoxia and death. If the patient lives, *microhaemorrhages* and *microinfarcts* may be seen in the brain.

The diagnosis of malaria is made by demonstration of malarial parasite in thin or thick blood films or sometimes in histologic sections (COLOUR PLATE XI: CL 43).

FILARIASIS

Wuchereria bancrofti and *Brugia malayi* are responsible for causing Bancroftian and Malayan filariasis in different geographic regions. The lymphatic vessels inhabit the adult worm, especially in the lymph nodes, testis and epididymis. Microfilariae seen in the circulation are produced by the female worm. Majority of infected patients remain asymptomatic. Symptomatic cases may have two forms of disease—an acute form and a chronic form.

■ *Acute form* of filariasis presents with fever, lymph-angitis, lymphadenitis, epididymo-orchitis, urticaria, eosinophilia and microfilariaemia.

■ *Chronic form* of filariasis is characterised by lymph-adenopathy, lymphoedema, hydrocele and elephantiasis.

The most significant histologic changes are due to the presence of adult worms in the lymphatic vessels causing lymphatic obstruction and lymphoedema. The regional lymph nodes are enlarged and their sinuses are distended with lymph. The tissues surrounding the blocked lymphatics are infiltrated by chronic inflammatory cell infiltrate consisting of lymphocytes, histiocytes, plasma cells and eosinophils. Chronicity of the process causes enormous thickening and induration of

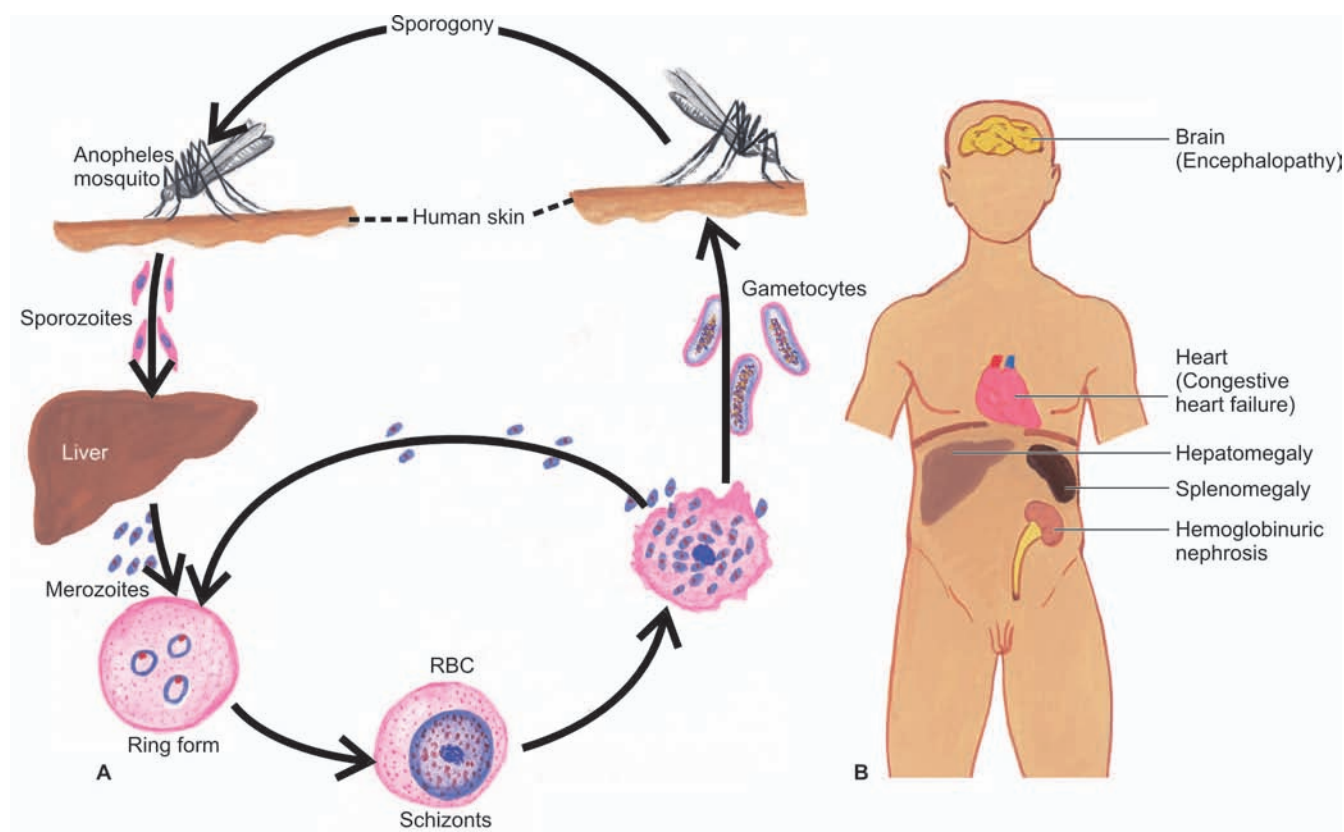


FIGURE 14.8

Life cycle of malaria (A) and major pathological changes in organs (B)

the skin of legs and scrotum resembling the hide of an elephant and hence the name *elephantiasis*. *Chylous ascites* and *chyluria* may occur due to rupture of the abdominal lymphatics.

CYSTICERCOSIS

Cysticercosis is infection by the larval stage of *Taenia solium*, the pork tapeworm. The adult tape-worm resides in the human intestines. The eggs are passed in human faeces which are ingested by pigs or they infect vegetables. These eggs then develop into larval stages in the host, spread by blood to any site in the body and form cystic larvae termed *cysticercus cellulosae*. Human beings may acquire infection by the larval stage by eating undercooked pork ('measly pork'), by ingesting uncooked contaminated vegetables, and sometimes, by autoinfection.

PATHOLOGIC CHANGES. The cysticercus may be single or there may be multiple cysticerci in the

different tissues of the body. The cysts may occur virtually anywhere in body and accordingly produce symptoms; most common sites are the brain, skeletal muscle and skin. The cysticercus consists of a round to oval white cyst, about 1 cm in diameter, contains milky fluid and invaginated scolex with birefringent hooklets. The cysticercus may remain viable for a long time and incite no inflammation. But when the embryo dies, it produces granulomatous reaction with eosinophils. Later, the lesion may become scarred and calcified (**COLOUR PLATE V: CL 18**).

HYDATID DISEASE (ECHINOCOCCOSIS)

Hydatid disease occurs as a result of infection by the larval cyst stage of the tapeworm, *Echinococcus granulosus*. The dog is the common definite host, while man, sheep and cattle are the intermediate hosts. The dog is infected by eating the viscera of sheep containing hydatid cysts. The infected faeces of the dog contaminate grass and farmland from where the ova are ingested by

sheep, pigs and man. Thus, man can acquire infection by handling dogs as well as by eating contaminated vegetables. The ova ingested by man are liberated from the chitinous wall by gastric juice and pass through the intestinal mucosa from where they are carried to the liver by portal venous system. These are trapped in the hepatic sinusoids where they eventually develop into hydatid cyst. About 70% of hydatid cysts develop in the liver which acts as the first filter for ova. However, ova which pass through the liver enter the right side of the heart and are caught in the pulmonary capillary bed and form pulmonary hydatid cysts. Some ova which enter the systemic circulation give rise to hydatid cysts in the brain, spleen, bone and muscles.

The disease is common in sheep-raising countries such as Australia, New Zealand and South America. The *uncomplicated hydatid cyst* of the liver may be silent or may produce dull ache in the liver area and some abdominal distension.

Complications of hydatid cyst include its rupture (e.g. into the peritoneal cavity, bile ducts and lungs), secondary infection and hydatid allergy due to sensitisation of the host with cyst fluid. The diagnosis is made by peripheral blood eosinophilia, radiologic examination and serologic tests such as indirect haemagglutination test and Casoni skin test.

PATHOLOGIC CHANGES. Hydatid cyst grows slowly and may eventually attain a size over 10 cm in diameter in about 5 years. *E. granulosus* generally causes unilocular hydatid cyst while *E. multilocularis*

results in multilocular or alveolar hydatid disease in the liver.

The cyst wall is composed of 3 distinguishable zones—outer *pericyst*, intermediate characteristic *ectocyst* and inner *endocyst* (Fig. 14.9,A,B) (**COLOUR PLATE V: CL 20**):

1. **Pericyst** is the outer host inflammatory reaction consisting of fibroblastic proliferation, mononuclear cells, eosinophils and giant cells, eventually developing into dense fibrous capsule which may even calcify.

2. **Ectocyst** is the intermediate layer composed of characteristic acellular, chitinous, laminated hyaline material.

3. **Endocyst** is the inner germinal layer bearing daughter cysts (brood-capsules) and scolices projecting into the lumen.

Hydatid sand is the grain-like material composed of numerous scolices present in the hydatid fluid. Hydatid fluid, in addition, contains antigenic proteins so that its liberation into circulation gives rise to pronounced eosinophilia or may cause anaphylaxis.

TORCH COMPLEX

TORCH complex refers to development of common complex of symptoms in infants due to infection with different microorganisms that include: *Toxoplasma*, *Rubella*, *Cytomegalovirus*, and *Herpes simplex virus*. The infection may be acquired by the foetus during intrauterine life, or perinatally and damage the foetus

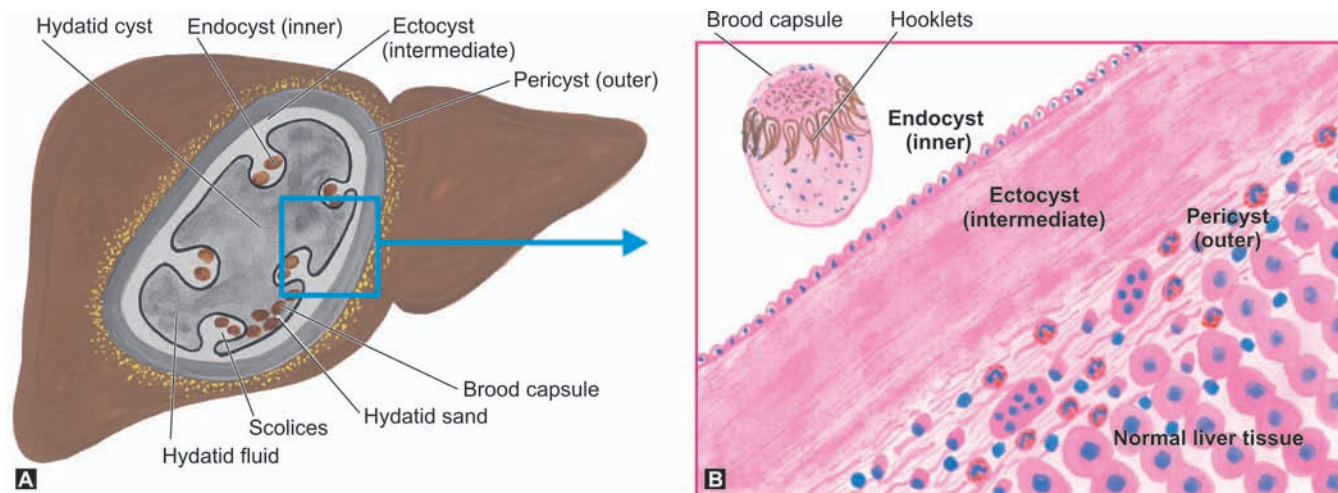


FIGURE 14.9

A, Hydatid cyst in the liver. B, Microscopy shows three layers in the wall of hydatid cyst.

or infant. Since the symptoms produced by TORCH group of organisms are indistinguishable from each other, it is a common practice in a suspected pregnant mother or infant to test for all the four main TORCH agents.

It has been estimated that TORCH complex infections have an overall incidence of 1-5% of all live born children. All the microorganisms in the TORCH complex are transmitted transplacentally and, therefore, infect the foetus from mother. Herpes and cytomegalovirus infections are common intrapartum infections acquired venereally. An infectious mononucleosis-like disease is present in about 10% of mothers whose infants have *Toxoplasma* infection. Genital herpes infection is present in 20% of mothers whose newborn babies suffer from herpes infection. Rubella infection during acute stage in the first 10 weeks of pregnancy is more harmful to the foetus than at later stage of gestation. Symptoms of cytomegalovirus infection are present in less than 1% of mothers who display antibodies to it.

The classic features of syndrome produced by TORCH complex are seen in congenital rubella. The features include: ocular defects, cardiac defects, CNS manifestations, sensorineural deafness, thrombocytopenia and hepatosplenomegaly (Fig. 14.10).

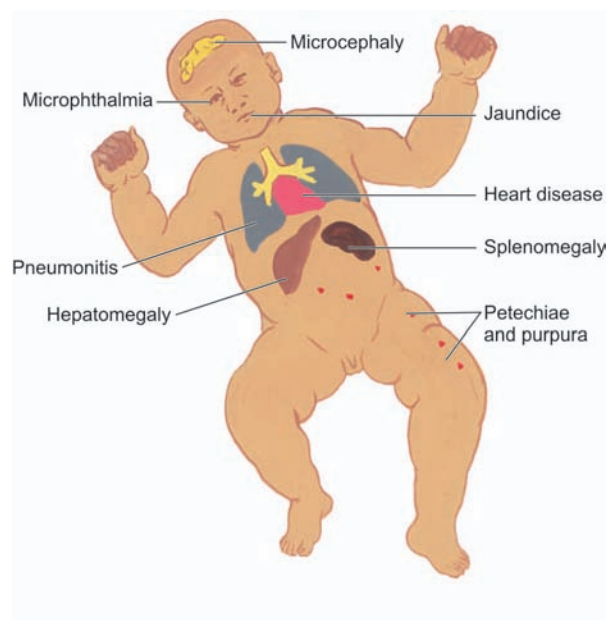


FIGURE 14.10

Lesions produced by TORCH complex infection in foetus *in utero*.

The foetal damage caused by TORCH complex infection is irreparable and, therefore, prevention is the best mode of therapy.



Fluid and Haemodynamic Disorders

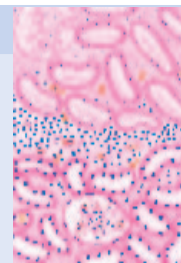
SECTION CONTENTS

CHAPTER 15: Derangements of Body Fluids and Electrolytes

CHAPTER 16: Circulatory Disturbances Due to Deranged Volume

CHAPTER 17: Circulatory Disturbances of Obstructive Nature

CHAPTER 18: Ischaemia and Infarction



15

Derangements of Body Fluids and Electrolytes

INTERNAL ENVIRONMENT

Many workers have pointed out that life on earth probably arose in the sea, and that the body water which is the environment of the cells, is similar to the ancient ocean from where life began. Although it appears quite tempting to draw comparison between environment of the cell and the ancient oceans but it would be rather an oversimplification in considering the cellular environment to be wholly fluid ignoring the presence of cells, fibres and ground substance.

Claude Bernarde (1949) first coined the term *internal environment* or *milieu interieur* for the state in the body in which the interstitial fluid that bathes the cells and the plasma, together maintain the normal morphology and function of the cells and tissues of the body. The mechanism by which the constancy of the internal environment is maintained and ensured is called the *homeostasis*. For this purpose, living membranes with varying permeabilities such as vascular endothelium and the cell wall play important role in exchange of fluids, electrolytes, nutrients and metabolites across the compartments of body fluids.

The normal composition of internal environment consists of the following components (Fig. 15.1):

1. WATER. Water is the principal and essential constituent of the body. The total body water in a normal adult male comprises 50-70% (average 60%) of the body weight and about 10% less in a normal adult female (average 50%). Thus, the body of a normal man weigh-

ing 65 kg contains approximately 40 litres of water. The total body water (assuming average of 60%) is distributed into 2 main compartments of body fluids separated from each other by membranes freely permeable to water. These are as under (Fig. 15.2):

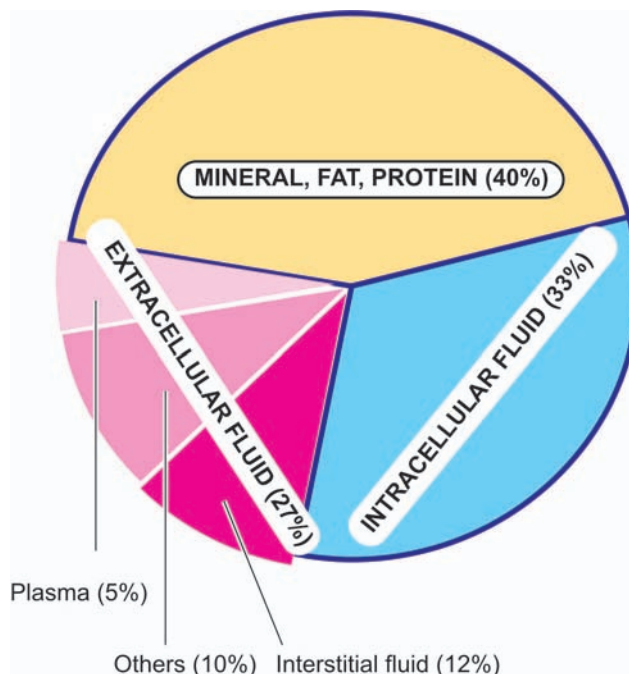


FIGURE 15.1

Body fluid compartments.

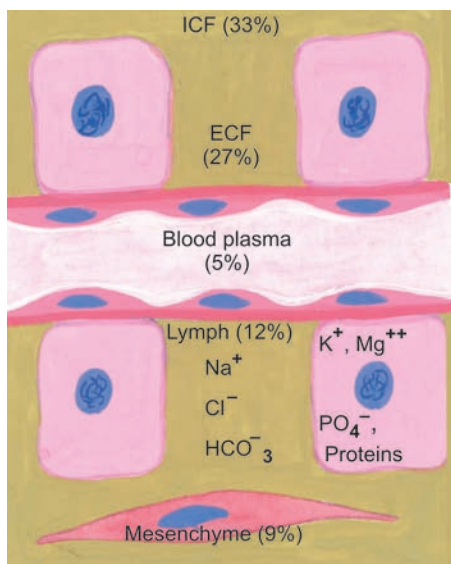


FIGURE 15.2

Body fluid compartments. (ICF = intracellular fluid compartment; ECF = extracellular fluid compartment).

i) Intracellular fluid compartment. This comprises about 33% of the body weight, the bulk of which is contained in the muscles.

ii) Extracellular fluid compartment. This constitutes the remaining 27% of body weight containing water. Included in this are the following 4 subdivisions of extracellular fluid (ECF):

- Interstitial fluid including lymph fluid* constitutes the major proportion of ECF (12% of body weight).
- Intravascular fluid or blood plasma* comprises about 5% of the body weight. Thus plasma content is about 3 litres of fluid out of 5 litres of total blood volume.
- Mesenchymal tissues* such as dense connective tissue, cartilage and bone contain body water that comprises about 9% of the body weight.
- Transcellular fluid* constitutes 1% of body weight. This is the fluid contained in the secretions of secretory cells of the body e.g. skin, salivary glands, mucous membranes of alimentary and respiratory tracts, pancreas, liver and biliary tract, kidneys, gonads, thyroid, lacrimal gland and CSF.

2. ELECTROLYTES. The concentration of cations (positively charged) and anions (negatively charged) is different in intracellular and extracellular fluids:

■ *In the intracellular fluid*, the main cations are potassium and magnesium and the main anions are

phosphates and proteins. It has low concentration of sodium and chloride.

■ *In the extracellular fluid*, the predominant cation is sodium and the principal anions are chloride and bicarbonate. Besides these, a small proportion of non-diffusible proteins and some diffusible nutrients and metabolites such as glucose and urea are present in the ECF.

The essential difference between the two main subdivisions of ECF is the higher protein content in the plasma than in the interstitial fluid which plays an important role in maintaining fluid balance.

The **major functions** of electrolytes are as follows:

- Electrolytes are the main solutes in the body fluids for maintenance of acid-base equilibrium.
- Electrolytes maintain the proper osmolality and volume of body fluids (*Osmolality* is the solute concentration per kg water compared from *osmolarity* which is the solute concentration per litre solution).
- The concentration of certain electrolytes determines their specific physiologic functions e.g. the effect of calcium ions on neuromuscular excitability. The concentration of the major electrolytes is expressed in milliequivalent (mEq) per litre so as to compare the values directly with each other. In order to convert mg per dl into mEq per litre the following formula is used:

$$\text{mEq/L} = \frac{\text{mg/dl}}{\text{Eq weight of element}} \times 10$$

NORMAL WATER AND ELECTROLYTE BALANCE (GIBBS-DONNAN EQUILIBRIUM)

Normally, a state of balance exists between the amount of water absorbed into the body and that which is eliminated from the body. The water as well as electrolytes are distributed nearly constantly in different body fluid compartments.

1. Water is normally *absorbed* into the body from the bowel or is introduced parenterally; average intake being 2800 ml per day.

2. Water is *eliminated* from the body via:

- kidneys in the urine (average 1500 ml per day);
- via the skin as insensible loss in perspiration or as sweat (average 800 ml per day), though there is wide variation in loss via sweat depending upon weather, temperature, fever and exercise;
- via the lungs in exhaled air (average 400 ml per day); and
- minor losses via the faeces (average 100 ml per day) and lacrimal, nasal, oral, sexual and mammary (milk) secretions.

The cell wall as well as capillary endothelium are entirely permeable to water but they differ in their permeability to electrolytes. Capillary wall is completely permeable to electrolytes while the cell membrane is somewhat impermeable. As mentioned earlier, concentration of potassium and phosphate are high in the intracellular fluid whereas concentration of sodium and chloride are high in the ECF. The osmotic equilibrium between the two major body fluid compartments is maintained by the passage of water from or into the intracellular compartment. The 2 main subdivisions of ECF—blood plasma and interstitial fluid, are separated from each other by capillary wall which is freely permeable to water but does not allow free passage of macromolecules of plasma proteins resulting in higher protein content in the plasma.

ACID-BASE BALANCE

Besides changes in the volume of fluids in the compartments, changes in ionic equilibrium affecting the acid-base balance of fluids occur. In terms of body fluids,

- an *acid* is a molecule or ion which is capable of giving off a hydrogen ion (H^+ ion donor); and
- a *base* is a molecule or ion which is capable of taking up hydrogen ion (H^+ ion acceptor).

A number of acids such as carbonic, phosphoric, sulfuric, lactic, hydrochloric and ketoacids are formed during normal metabolic activity. However, carbonic acid is produced in largest amount as it is the end-product of aerobic tissue activity. In spite of these acids, the pH of the blood is kept constant at 7.4 ± 0.05 in health.

The pH of blood and acid-base balance are regulated in the body by the following factors:

1. BUFFER SYSTEM. Buffers are substances which have weak acids and strong bases and limit the change in H^+ ion concentration to the normal range. They are the first line of defense for maintaining acid-base balance and do so by taking up H^+ ions when the pH rises. The most important buffer which regulates the pH of blood is *bicarbonate-carbonic acid system* followed by *intracellular buffering action of haemoglobin* and *carbonic anhydrase* in the red cells.

2. PULMONARY MECHANISM. During respiration, CO_2 is removed by the lungs depending upon the partial pressure of CO_2 in the arterial blood. With ingestion of high quantity of acid-forming salts, ventilation is increased as seen in acidosis in diabetic ketosis and uraemia.

3. RENAL MECHANISM. The other route by which H^+ ions can be excreted from the body is in the urine. Here H^+ ions secreted by the renal tubular cells are buffered in the glomerular filtrate by:

- *combining with phosphates* to form phosphoric acid;
- *combining with ammonia* to form ammonium ions; and
- *combining with filtered bicarbonate ions* to form carbonic acid.

However, carbonic acid formed is dissociated to form CO_2 which diffuses back into the blood to reform bicarbonate ions.

PRESSURE GRADIENTS AND FLUID EXCHANGES

Aside from water and electrolytes or crystalloids, both of which are freely interchanged between the interstitial fluid and plasma, the ECF contains colloids like proteins which minimally cross the capillary wall. These substances exert pressures responsible for exchange between the interstitial fluid and plasma.

Normal Fluid Pressures (Fig. 15.3,A)

1. OSMOTIC PRESSURE. This is the pressure exerted by the chemical constituents of the body fluids. Accordingly, osmotic pressure may be of the following types:

■ **Crystalloid osmotic pressure** exerted by electrolytes present in the ECF and comprises the major portion of the total osmotic pressure.

■ **Colloid osmotic pressure or oncotic pressure** exerted by proteins present in the ECF and constitutes a small part of the total osmotic pressure but is more significant physiologically. Since the protein content of the plasma is higher than that of interstitial fluid, oncotic pressure of plasma is higher (average 25 mmHg) than that of interstitial fluid (average 8 mmHg).

■ **Effective oncotic pressure** is the difference between the higher oncotic pressure of plasma and the lower oncotic pressure of interstitial fluid and is *the force that tends to draw fluid into the vessels*.

2. HYDROSTATIC PRESSURE. This is the capillary blood pressure.

There is considerable *pressure gradient* at the two ends of capillary loop—being higher at the arteriolar end (average 32 mmHg) than at the venular end (average 12 mmHg).

■ **Tissue tension** is the hydrostatic pressure of interstitial fluid and is lower than the hydrostatic pressure in the capillary at either end (average 4 mmHg).

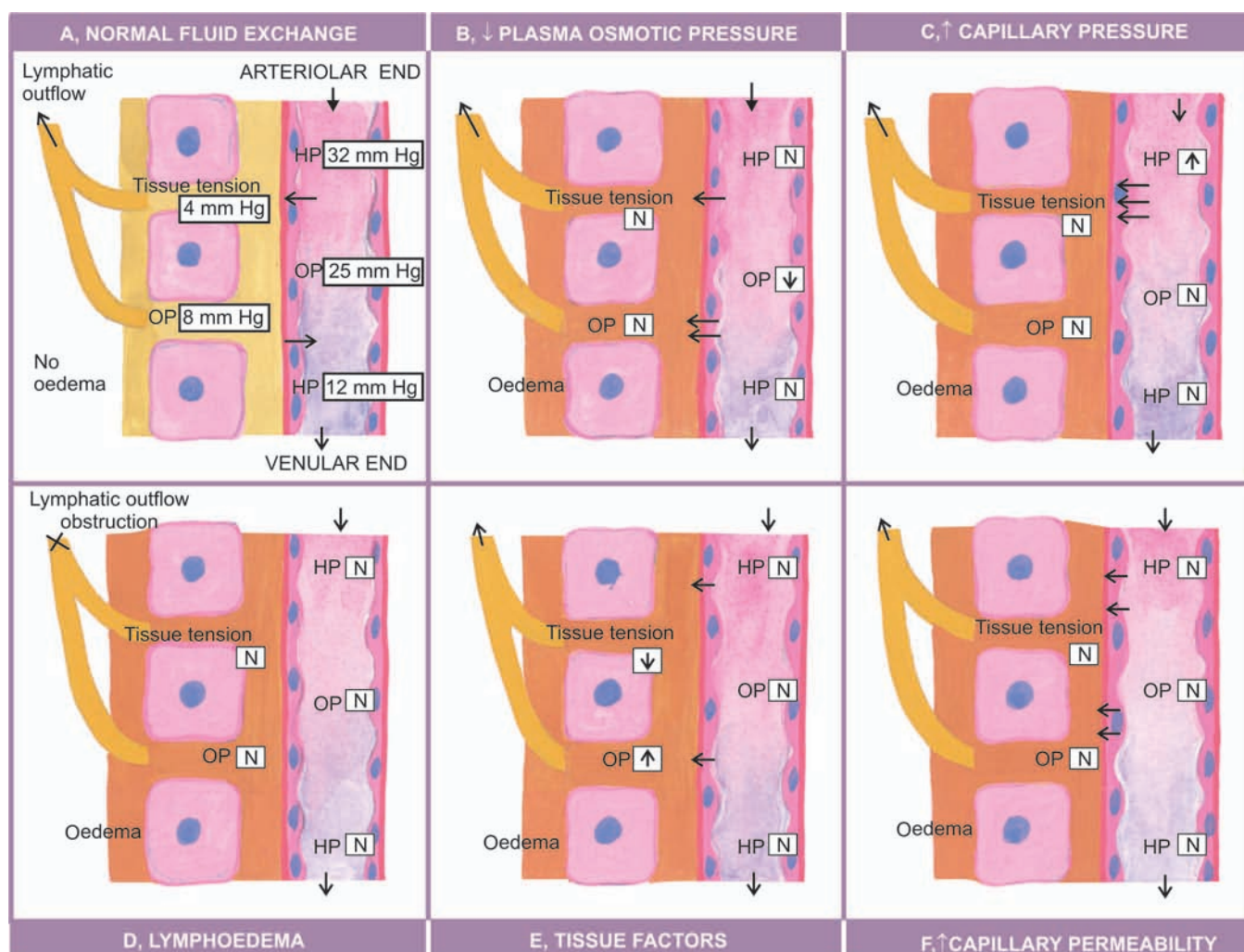


FIGURE 15.3

Diagrammatic representation of pathogenesis of oedema (OP = oncotic pressure; HP = hydrostatic pressure). A, Normal pressure gradients and fluid exchanges between plasma, interstitial space and lymphatics. B, Mechanism of oedema by decreased plasma oncotic pressure and hypoproteinaemia. C, Mechanism of oedema by increased hydrostatic pressure in the capillary. D, Mechanism of lymphoedema. E, Mechanism by tissue factors (increased oncotic pressure of interstitial fluid and lowered tissue tension). F, Mechanism of oedema by increased capillary permeability.

■ **Effective hydrostatic pressure** is the difference between the higher hydrostatic pressure in the capillary and the lower tissue tension; it is *the force that drives fluid through the capillary wall into the interstitial space*.

Normal Fluid Exchanges

Normally, the fluid exchanges between the body compartments take place as under (Fig. 15.3,A):

■ *At the arteriolar end of the capillary*, the balance between the hydrostatic pressure (32 mmHg) and plasma oncotic pressure (25 mmHg) is the hydrostatic

pressure of 7 mmHg which is the outward-driving force so that a small quantity of fluid and solutes leave the vessel to enter the interstitial space.

■ *At the venular end of the capillary*, the balance between the hydrostatic pressure (12 mmHg) and plasma oncotic pressure (25 mmHg) is the oncotic pressure of 13 mmHg which is the inward-driving force so that the fluid and solutes re-enter the plasma.

■ *The tissue fluid left after exchanges across the capillary wall escapes into the lymphatics from where it is finally drained into venous circulation.*

■ *Tissue factors* i.e. oncotic pressure of interstitial fluid and tissue tension, are normally small and insignificant forces opposing the plasma hydrostatic pressure and capillary hydrostatic pressure, respectively.

DISTURBANCES OF BODY FLUIDS AND ELECTROLYTES

The common derangements of body fluid are as follows:

1. Oedema;
 2. Overhydration; and
 3. Dehydration or pure water deficiency.
- These are discussed below.

OEDEMA

DEFINITION AND TYPES

The Greek word *oidema* means swelling. *Oedema may be defined as abnormal and excessive accumulation of fluid in the interstitial tissue spaces and serous cavities.* The presence of abnormal collection of fluid within the cell is sometimes called intracellular oedema but should more appropriately be called hydropic degeneration (page 33). The accumulation of fluid in the body cavities is correspondingly known as ascites (in the peritoneal cavity), hydrothorax or pleural effusion (in the pleural cavity), and hydropericardium or pericardial effusion (in the pericardial cavity). The oedema may be of 2 main types (Fig. 15.4):

1. *Localised* in the organ or limb; and
2. *Generalised* (*anasarca* or *dropsy*) when it is systemic in distribution, particularly noticeable in the subcutaneous tissues.

Besides, there are a few special forms of oedema.

The oedema fluid lies free in the interstitial space between the cells and can be displaced from one place to another. In the case of oedema in the subcutaneous tissues, momentary pressure of finger produces a depression known as *pitting oedema*. The other variety is *non-pitting* or *solid oedema* in which no pitting is pro-

duced on pressure e.g. in myxoedema, elephantiasis. Oedema fluid may be:

- *transudate* which is more often the case, such as in oedema of cardiac and renal disease; or
- *exudate* such as in inflammatory oedema.

The differences between transudate and exudate are tabulated in Table 15.1.

PATHOGENESIS OF OEDEMA

Oedema is caused by mechanisms that interfere with normal fluid balance of plasma, interstitial fluid and lymph flow. The following six mechanisms may be operating singly or in combination to produce oedema:

1. Decreased plasma oncotic pressure
2. Increased capillary hydrostatic pressure
3. Lymphatic obstruction
4. Tissue factors (increased oncotic pressure of interstitial fluid, and decreased tissue tension)
5. Increased capillary permeability
6. Sodium and water retention.

These mechanisms are discussed below and illustrated in Fig. 15.3:

1. DECREASED PLASMA ONCOTIC PRESSURE.

The plasma oncotic pressure exerted by the total amount of plasma proteins tends to draw fluid into the vessels normally. A fall in the total plasma protein level (hypoproteinaemia of less than 5 g/dl), results in lowering of plasma oncotic pressure in a way that it can no longer counteract the effect of hydrostatic pressure of blood. This results in increased outward movement of fluid from the capillary wall and decreased inward movement of fluid from the interstitial space causing oedema (Fig. 15.3,B). Hypoproteinaemia usually produces generalised oedema (anasarca). Out of the various plasma proteins, albumin has four times higher plasma oncotic pressure than globulin so that it is hypoalbuminaemia (albumin below 2.5 g/dl) that results in oedema more often.

The **examples** of oedema by this mechanism are seen in the following conditions:

- i) *Oedema of renal disease* e.g. in nephrotic syndrome, acute glomerulonephritis.
- ii) *Ascites of liver disease* e.g. in cirrhosis.
- iii) *Oedema due to other causes of hypoproteinaemia* e.g. in protein-losing enteropathy.

2. INCREASED CAPILLARY HYDROSTATIC PRESSURE.

The hydrostatic pressure of the capillary is the force that normally tends to drive fluid through the capillary wall into the interstitial space by counteracting the force of plasma oncotic pressure. A rise in

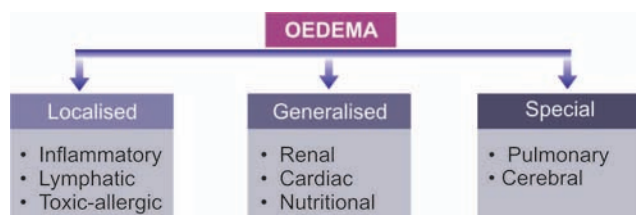


FIGURE 15.4

Types of oedema.

TABLE 15.1: Differences between Transudate and Exudate.

FEATURE	TRANSUDATE	EXUDATE
1. <i>Definition</i>	Filtrate of blood plasma without changes in endothelial permeability	Oedema of inflamed tissue associated with increased vascular permeability
2. <i>Character</i>	Non-inflammatory oedema	Inflammatory oedema
3. <i>Protein content</i>	Low (less than 1 g/dl); mainly albumin, low fibrinogen; hence no tendency to coagulate	High (2.5-3.5 g/dl), readily coagulates due to high content of fibrinogen and other coagulation factors
4. <i>Glucose content</i>	Same as in plasma	Low (less than 60 mg/dl)
5. <i>Specific gravity</i>	Low (less than 1.015)	High (more than 1.018)
6. <i>pH</i>	> 7.3	< 7.3
7. <i>LDH</i>	Low	High
8. <i>Effusion LDH/ Serum LDH ratio</i>	< 0.6	> 0.6
9. <i>Cells</i>	Few cells, mainly mesothelial cells and cellular debris	Many cells, inflammatory as well as parenchymal
10. <i>Examples</i>	Oedema in congestive cardiac failure	Purulent exudate such as pus

the hydrostatic pressure at the venular end of the capillary which is normally low (average 12 mmHg) to a level more than the plasma oncotic pressure results in minimal or no reabsorption of fluid at the venular end, consequently leading to oedema (Fig. 15.3,C).

The **examples** of oedema by this mechanism are seen in the following disorders:

- i) *Oedema of cardiac disease* e.g. in congestive cardiac failure, constrictive pericarditis.
- ii) *Ascites of liver disease* e.g. in cirrhosis of liver.
- iii) *Passive congestion* e.g. in mechanical obstruction due to thrombosis of veins of the lower legs, varicosities, pressure by pregnant uterus, tumours etc.
- iv) *Postural oedema* e.g. transient oedema of feet and ankles due to increased venous pressure seen in individuals who remain standing erect for longtime such as traffic constables.

3. LYMPHATIC OBSTRUCTION. Normally the interstitial fluid in the tissue spaces escapes by way of lymphatics so that obstruction to outflow of these channels causes localised oedema, known as lymphoedema (Fig. 15.3,D).

The **examples** of lymphoedema include the following:

- i) *Removal of axillary lymph nodes* in radical mastectomy for carcinoma of the breast produces lymphoedema of the affected arm.
- ii) *Pressure from outside* on the main abdominal or thoracic duct such as due to tumours, effusions in serous cavities etc may produce lymphoedema. At times, the main lymphatic channel may rupture and discharge

chyle into the pleural cavity (chylothorax) or into peritoneal cavity (chylous ascites).

iii) *Inflammation of the lymphatics* as seen in filariasis (infection with *Wuchereria bancrofti*) results in chronic lymphoedema of scrotum and legs known as elephantiasis.

iv) *Occlusion of lymphatic channels* by malignant cells may result in lymphoedema.

v) *Milroy's disease or hereditary lymphoedema* is due to abnormal development of lymphatic channels. It is seen in families and the oedema is mainly confined to one or both the lower limbs.

4. TISSUE FACTORS. The forces acting in the interstitial space—oncotic pressure of the interstitial space and tissue tension, are normally quite small and insignificant to counteract the effects of plasma oncotic pressure and capillary hydrostatic pressure respectively. However, in some situations, the tissue factors in combination with other mechanisms play a role in causation of oedema (Fig. 15.3,E). These are as under:

- i) *Elevation of oncotic pressure of interstitial fluid* as occurs due to increased vascular permeability and inadequate removal of proteins by lymphatics.
- ii) *Lowered tissue tension* as seen in *loose subcutaneous tissues* of eyelids and external genitalia.

5. INCREASED CAPILLARY PERMEABILITY. As described previously, an intact capillary endothelium is a semipermeable membrane which permits the free flow of water and crystalloids but allows minimal passage of plasma proteins normally. However, when the capillary endothelium is injured by various 'capillary

poisons' such as toxins and their products, histamine, anoxia, venoms, certain drugs and chemicals, the capillary permeability to plasma proteins is enhanced due to development of gaps between the endothelial cells. This, in turn, causes reduced plasma oncotic pressure and elevated oncotic pressure of interstitial fluid which consequently produces oedema (Fig. 15.3,F).

The **examples** of oedema by this mechanism are seen in the following conditions:

- i) *Generalised oedema* due to increased vascular permeability may occur in systemic infections, poisonings, certain drugs and chemicals, anaphylactic reactions and anoxia.
- ii) *Localised oedema* such as:
 - *Inflammatory oedema* as seen in infections, allergic reactions, insect-bite, irritant drugs and chemicals. It is generally exudate in nature.
 - *Angioneurotic oedema* is an acute attack of localised oedema occurring on the skin of face and trunk and may involve lips, larynx, pharynx and lungs. It is possibly neurogenic or allergic in origin.

6. SODIUM AND WATER RETENTION. Before describing the mechanism of oedema by sodium and water retention, it is essential to recollect the normal regulatory mechanism of sodium and water balance.

Normally, about 80% of sodium is reabsorbed by the proximal convoluted tubule under the influence of intrinsic renal mechanism or extra-renal mechanism:

■ **Intrinsic renal mechanism** is activated in response to sudden reduction in the effective arterial blood volume (hypovolaemia) as occurs in severe haemorrhage. Hypovolaemia stimulates the arterial baroreceptors present in the carotid sinus and aortic arch which, in turn, send the sympathetic outflow via the vasomotor centre in the brain. As a result of this, renal ischaemia occurs which causes reduction in the glomerular filtration rate, decreased excretion of sodium in the urine and consequent retention of sodium.

■ **Extra-renal mechanism** involves the secretion of aldosterone, a sodium retaining hormone, by the *renin-angiotensin-aldosterone system*. Renin is an enzyme secreted by the granular cells in the juxta-glomerular apparatus. Its release is stimulated in response to low concentration of sodium in the tubules. Its main action is stimulation of the angiotensinogen which is α_2 -globulin or renin substrate present in the plasma. On stimulation, angiotensin I, a decapeptide, is formed in the plasma which is subsequently converted into angiotensin II, an octapeptide, in the lungs and kidneys. Angiotensin II stimulates the adrenal cortex to secrete aldosterone hormone. Aldosterone increases sodium

reabsorption in the renal tubules and sometimes causes a rise in the blood pressure.

■ **ADH mechanism.** Retention of sodium leads to retention of water secondarily under the influence of anti-diuretic hormone (ADH) or vasopressin. This hormone is secreted by the cells of the supraoptic and paraventricular nuclei in the hypothalamus and is stored in the neurohypophysis (posterior pituitary). The release of hormone is stimulated by increased concentration of sodium in the plasma and hypovolaemia. Large amounts of ADH produce highly concentrated urine.

Excessive retention of sodium and water and their decreased renal excretion occur in response to hypovolaemia and lowered concentration of sodium in the renal tubules via stimulation of intrinsic renal and extra-renal mechanisms as well as via release of ADH (Fig. 15.5).

The **examples** of oedema by these mechanisms are as under:

- i) *Oedema of cardiac disease* e.g. in congestive cardiac failure.
- ii) *Ascites* of liver disease e.g. in cirrhosis of liver.
- iii) *Oedema of renal disease* e.g. in nephrotic syndrome, glomerulonephritis.

PATHOGENESIS AND MORPHOLOGY OF IMPORTANT TYPES OF OEDEMA

As observed from the pathogenesis of oedema just described, more than one mechanism may be involved in many examples of localised and generalised oedema. Some of the important examples are described below.

Renal Oedema

Generalised oedema occurs in certain diseases of renal origin such as in nephrotic syndrome, some types of glomerulonephritis, and in renal failure due to acute tubular injury.

1. Oedema in nephrotic syndrome. Since there is persistent and heavy proteinuria (albuminuria) in nephrotic syndrome, there is hypoalbuminaemia causing decreased plasma oncotic pressure resulting in severe generalised oedema (*nephrotic oedema*). The hypoalbuminaemia causes fall in the plasma volume activating renin-angiotensin-aldosterone mechanism which results in retention of sodium and water, thus setting in a vicious cycle which persists till the albuminuria continues. Similar type of mechanism operates in the pathogenesis of oedema in protein-losing enteropathy, further confirming the role of protein loss in the causation of oedema.

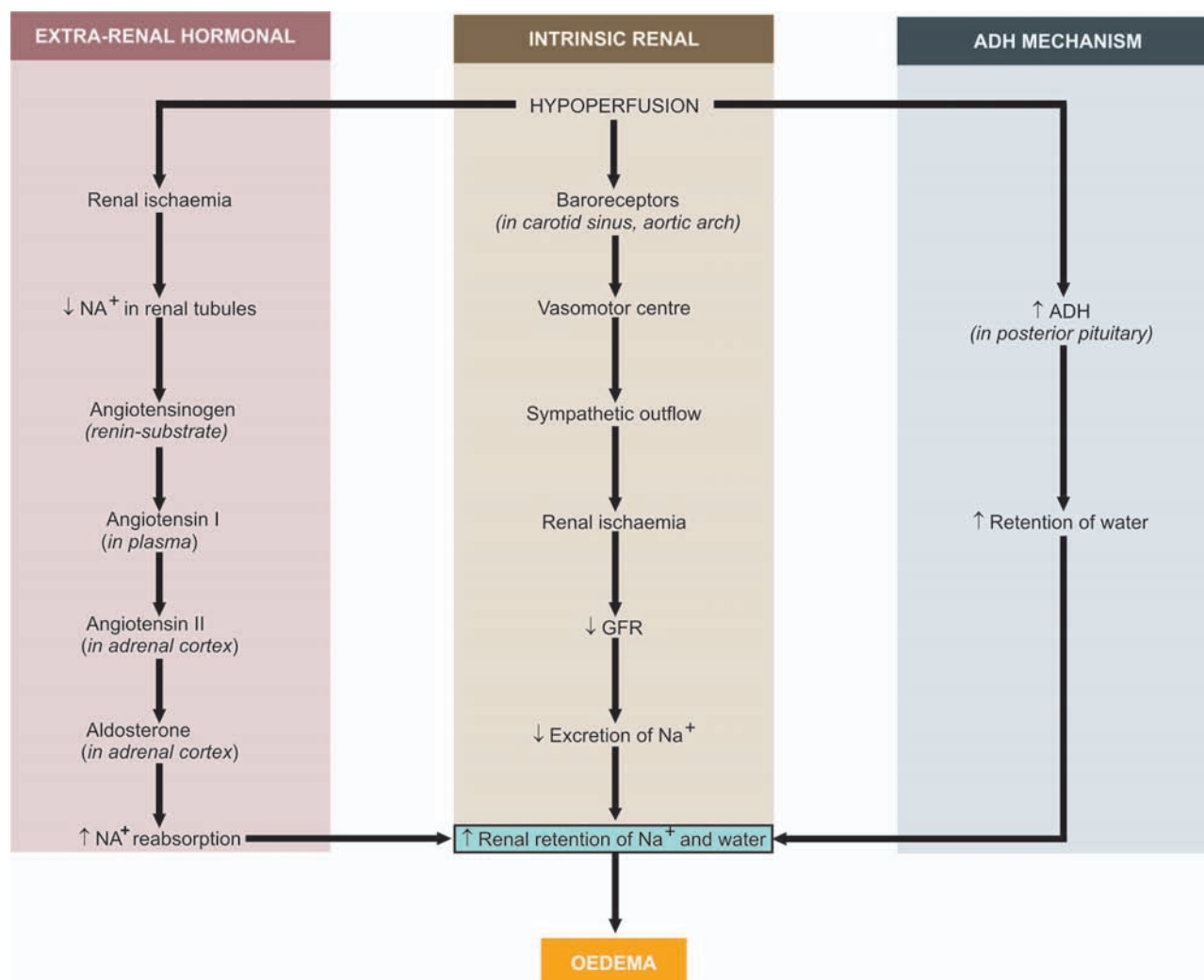


FIGURE 15.5

Mechanisms involved in oedema by sodium and water retention. On *left* and *middle* are the sequence of events in extra-renal hormonal and intrinsic-renal mechanisms respectively for sodium and water retention, whereas on *right* is shown the ADH mechanism for water retention.

The *nephrotic oedema* is classically more severe and marked and is present in the subcutaneous tissues as well as in the visceral organs. The affected organ is enlarged and heavy with tense capsule.

Microscopically, the oedema fluid separates the connective tissue fibres of subcutaneous tissues. Depending upon the protein content, the oedema fluid may appear homogeneous, pale, eosinophilic, or may be deeply eosinophilic and granular.

2. Oedema in glomerulonephritis. Oedema occurs in conditions with diffuse glomerular disease such as in acute diffuse glomerulonephritis and rapidly pro-

gressive glomerulonephritis (*nephritic oedema*). In contrast to nephrotic oedema, nephritic oedema is not due to hypoproteinaemia but is due to excessive reabsorption of sodium and water in the renal tubules via renin-angiotensin-aldosterone mechanism. The protein content of oedema fluid in glomerulonephritis is quite low (less than 0.5 g/dl).

The *nephritic oedema* is usually mild as compared to nephrotic oedema and begins in the loose tissues such as on the face around eyes, ankles and genitalia. Oedema in these conditions is usually not affected by gravity (unlike cardiac oedema).

The salient differences between the nephrotic and nephritic oedema are outlined in Table 15.2.

TABLE 15.2: Differences between Nephrotic and Nephritic Oedema.

FEATURE	NEPHROTIC OEDEMA	NEPHRITIC OEDEMA
1. Cause	Nephrotic syndrome	Glomerulonephritis (acute, rapidly progressive)
2. Proteinuria	Heavy	Moderate
3. Mechanism	↓ Plasma oncotic pressure, Na ⁺ and water retention	Na ⁺ and water retention
4. Degree of oedema	Severe, generalised	Mild
5. Distribution	Subcutaneous tissues as well as visceral organs	Loose tissues mainly (face, eyes, ankles, genitalia)

3. Oedema in acute tubular injury. Acute tubular injury following shock or toxic chemicals results in gross oedema of the body. The damaged tubules lose their capacity for selective reabsorption and concentration of the glomerular filtrate resulting in increased reabsorption and oliguria. Besides, there is excessive retention of water and electrolytes and rise in blood urea.

Cardiac Oedema

Generalised oedema develops in right-sided and congestive cardiac failure. Pathogenesis of cardiac

oedema is explained on the basis of the following hypotheses (Fig. 15.6):

1. Reduced cardiac output causes hypovolaemia which stimulates intrinsic-renal and extra-renal hormonal (renin-angiotensin-aldosterone) mechanisms as well as ADH secretion resulting in sodium and water retention and consequent oedema.
2. Due to heart failure, there is elevated central venous pressure which is transmitted backward to the venous end of the capillaries, raising the capillary hydrostatic pressure and consequent transudation; this is known as *back pressure hypothesis*.

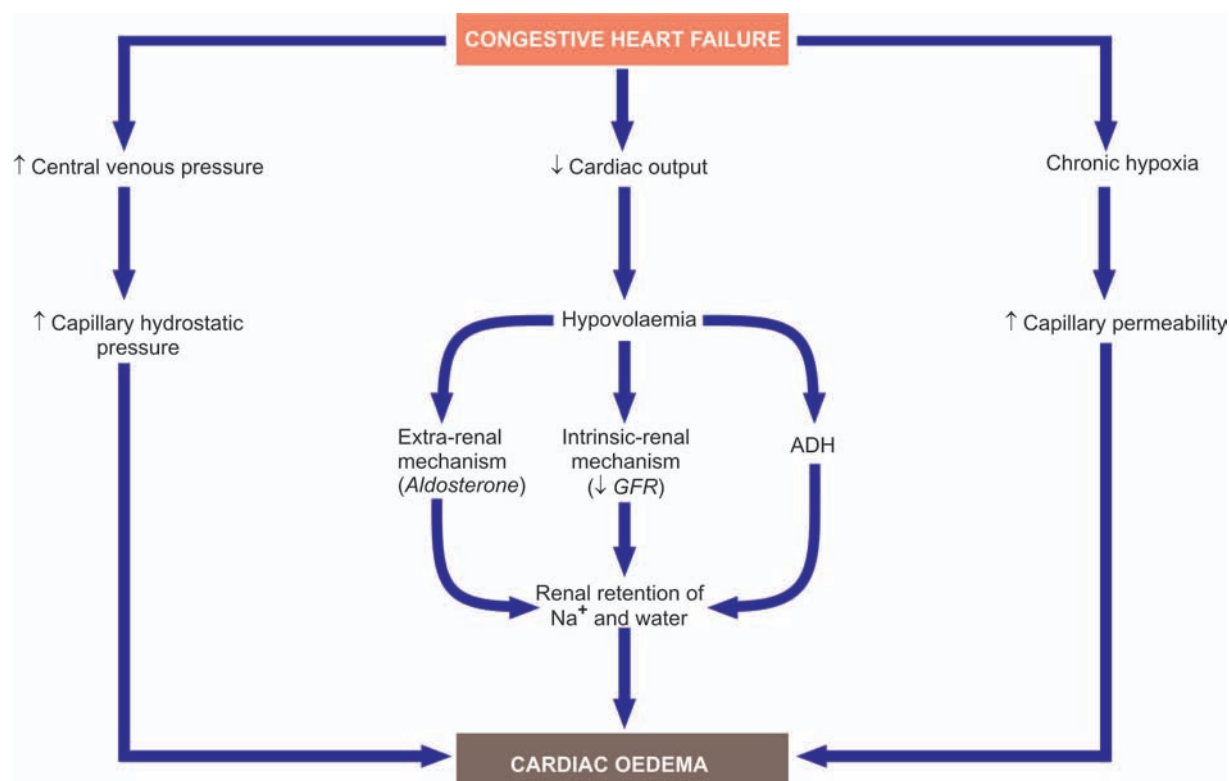


FIGURE 15.6

Mechanisms involved in the pathogenesis of cardiac oedema.

3. Chronic hypoxia may injure the capillary wall causing increased capillary permeability and result in oedema; this is called *forward pressure hypothesis*. However, this theory lacks support since the oedema by this mechanism is exudate whereas the cardiac oedema is typically transudate.

In left heart failure, the changes are, however, different. There is venous congestion, particularly in the lungs, so that pulmonary oedema develops rather than generalised oedema (described below).

Cardiac oedema is influenced by gravity and is thus characteristically *dependent oedema* i.e. in an ambulatory patient it is on the lower extremities, while in a bed-ridden patient oedema appears on the sacral and genital areas. The accumulation of fluid may also occur in serous cavities.

Pulmonary Oedema

Acute pulmonary oedema is the most important form of local oedema as it causes serious functional impairment but has special features. It differs from oedema elsewhere in that the fluid accumulation is not only in the tissue space but also in the pulmonary alveoli.

ETIOPATHOGENESIS. The hydrostatic pressure in the pulmonary capillaries is much lower (average 10 mmHg). Normally the plasma oncotic pressure is adequate to prevent the escape of fluid into the interstitial

space and hence lungs are normally free of oedema. Pulmonary oedema can result from either the elevation of pulmonary hydrostatic pressure or the increased capillary permeability (Fig. 15.7).

1. Elevation in pulmonary hydrostatic pressure (Haemodynamic oedema). In heart failure, there is increase in the pressure in pulmonary veins which is transmitted to pulmonary capillaries. This results in imbalance between pulmonary hydrostatic pressure and the plasma oncotic pressure so that excessive fluid moves out of pulmonary capillaries into the interstitium of the lungs. Simultaneously, the endothelium of the pulmonary capillaries develops fenestrations permitting passage of plasma proteins and fluid into the interstitium. The interstitial fluid so collected is cleared by the lymphatics present around the bronchioles, small muscular arteries and veins. As the capacity of the lymphatics to drain the fluid is exceeded (about ten-fold increase in fluid), the excess fluid starts accumulating in the interstitium (*interstitial oedema*) i.e. in the loose tissues around bronchioles, arteries and in the lobular septa. Next follows the thickening of the alveolar walls because of the interstitial oedema. Up to this stage, no significant impairment of gaseous exchange occurs. However, prolonged elevation of hydrostatic pressure and due to high pressure of interstitial oedema, the alveolar lining cells break and the alveolar air spaces

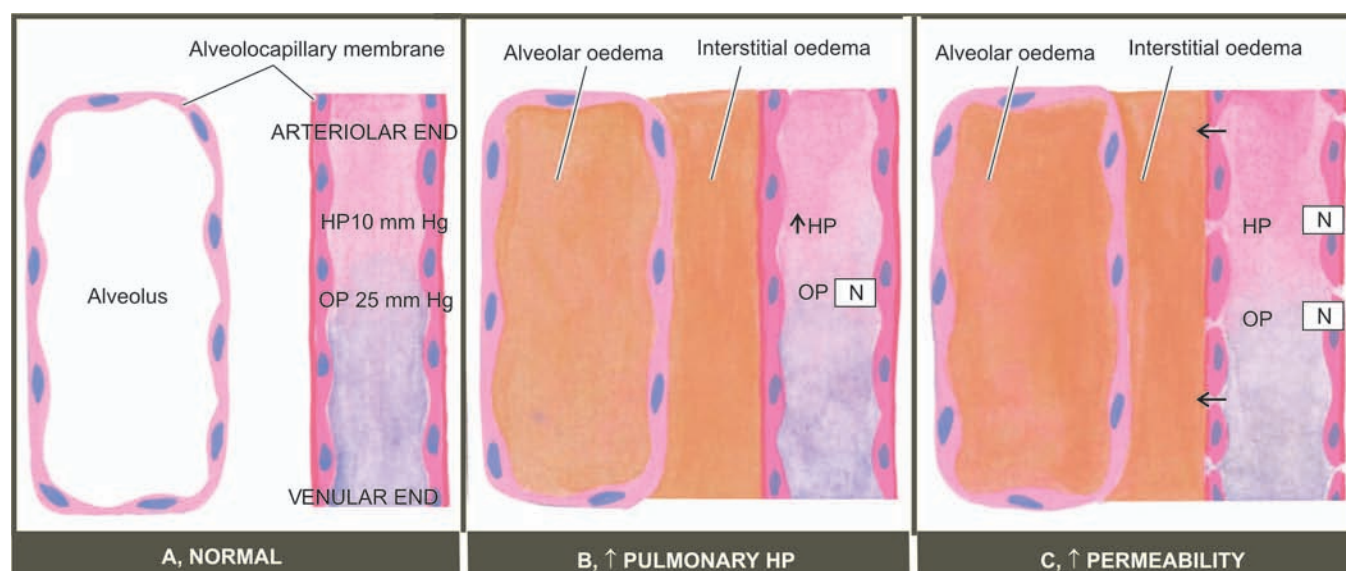


FIGURE 15.7

Mechanisms involved in the pathogenesis of pulmonary oedema. A, Normal fluid exchange at the alveolocapillary membrane (capillary endothelium and alveolar epithelium). B, Pulmonary oedema via elevated pulmonary hydrostatic pressure. C, Pulmonary oedema via increased vascular permeability.

are flooded with fluid (*alveolar oedema*) driving the air out of alveolus, thus seriously hampering the lung function.

Examples of pulmonary oedema by this mechanism are seen in left heart failure, mitral stenosis, pulmonary vein obstruction, thyrotoxicosis, cardiac surgery, nephrotic syndrome and obstruction to the lymphatic outflow by tumour or inflammation.

2. Increased vascular permeability (Irritant oedema).

The vascular endothelium as well as the alveolar epithelial cells (alveolo-capillary membrane) may be damaged causing increased vascular permeability so that excessive fluid and plasma proteins leak out, initially into the interstitium and subsequently into the alveoli.

This mechanism explains pulmonary oedema in *examples* such as in fulminant pulmonary and extrapulmonary infections, inhalation of toxic substances, aspiration, shock, radiation injury, hypersensitivity to drugs or antisera, uraemia and adult respiratory distress syndrome (ARDS).

3. Acute high altitude oedema. Individuals climbing to high altitude suddenly without halts and without waiting for acclimatisation to set in, suffer from serious circulatory and respiratory ill-effects. Commonly, the deleterious effects begin to appear after an altitude of 2500 metres is reached. These changes include: appearance of oedema fluid in the lungs, congestion and widespread minute haemorrhages. These changes can cause death within a few days. The underlying mechanism appears to be anoxic damage to the pulmonary vessels. However, if acclimatisation to high altitude is allowed to take place, the individual develops polycythaemia, raised pulmonary arterial pressure, increased pulmonary ventilation and a rise in heart rate and increased cardiac output.

PATHOLOGIC CHANGES. Irrespective of the underlying mechanism in the pathogenesis of pulmonary oedema, the fluid accumulates more in the basal regions of lungs. The thickened interlobular septa along with their dilated lymphatics may be seen in chest X-ray as linear lines perpendicular to the pleura and are known as *Kerley's lines*.

Grossly, the lungs in pulmonary oedema are heavy, moist and subcrepitant. Cut surface exudes frothy fluid (mixture of air and fluid).

Microscopically, the alveolar capillaries are congested. Initially the excess fluid collects in the interstitial lung spaces (interstitial oedema) but later the fluid fills the alveolar spaces (alveolar oedema). The interstitium as well as the alveolar spaces thus contain an

eosinophilic, granular and pink proteinaceous material, often admixed with some RBCs and macrophages (Fig. 15.8). This may be seen as brightly eosinophilic pink lines along the alveolar margin called *hyaline membrane*. Long-standing pulmonary oedema is prone to get infected by bacteria producing hypostatic pneumonia which may be fatal.

Cerebral Oedema

Cerebral oedema or swelling of brain is the most threatening example of oedema. The mechanism of fluid exchange in the brain differs from elsewhere in the body since there are no draining lymphatics in the brain but instead, the function of fluid-electrolyte exchange is performed by the blood-brain barrier located at the endothelial cells of the capillaries.

Cerebral oedema can be of 3 types:

1. VASOGENIC OEDEMA. This is the most common type and corresponds to oedema elsewhere resulting from increased filtration pressure or increased capillary permeability. Vasogenic oedema is prominent around cerebral contusions, infarcts, brain abscess and some tumours.

Grossly, the white matter is swollen, soft, with flattened gyri and narrowed sulci. Sectioned surface is soft and gelatinous.

Microscopically, there is separation of tissue elements by the oedema fluid and swelling of astrocytes. The

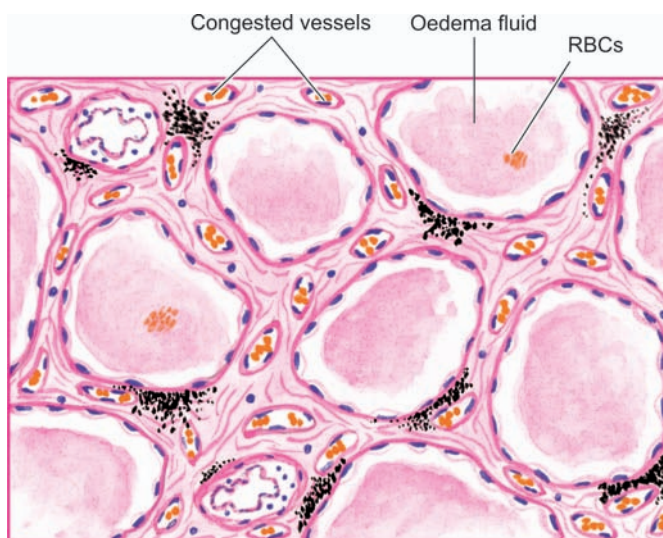


FIGURE 15.8

Pulmonary oedema. The alveolar capillaries are congested. The alveolar spaces as well as interstitium contain eosinophilic, granular, homogeneous and pink proteinaceous oedema fluid along with some RBCs and inflammatory cells.

perivascular (Virchow-Robin) space is widened and clear halos are seen around the small blood vessels.

2. CYTOTOXIC OEDEMA. In this type, the blood-brain barrier is intact and the fluid accumulation is intracellular. The underlying mechanism is disturbance in the cellular osmoregulation as occurs in some metabolic derangements, acute hypoxia and with some toxic chemicals.

Microscopically, the cells are swollen and vacuolated. In some situations, both vasogenic as well as cytotoxic cerebral oedema results e.g. in purulent meningitis.

3. INTERSTITIAL OEDEMA. This type of cerebral oedema occurs when the excessive fluid crosses the ependymal lining of the ventricles and accumulates in the periventricular white matter. This mechanism is responsible for oedema in non-communicating hydrocephalus.

OVERHYDRATION

Overhydration is a state of pure water excess or water intoxication.

PATHOGENESIS. A normal healthy individual can consume large volumes of water and respond via water diuresis without producing any deleterious effects. This is because the normal individual has the capacity to excrete large volumes of dilute urine when excess of water is given without electrolytes. However, in certain conditions, the kidneys have a restricted ability to dilute the urine when large amounts of pure water are given. This results in expansion of extracellular fluid compartment with reduced osmolality and intracellular oedema. An early diuretic phase is followed by oliguria or even anuria due to damaged renal tubular epithelial cells.

CAUSES. Overhydration is encountered in the following conditions:

1. Acute and chronic renal disease
2. Severe congestive heart failure
3. Addison's disease
4. Cirrhosis of the liver
5. Tumours producing ADH e.g. bronchogenic carcinoma
6. Early postoperative period when patient is on infusion of glucose solution but is incapable of diluting the urine due to liberation of vasopressin (ADH) by the stress of surgery.

CLINICAL AND BIOCHEMICAL EFFECTS. These are as under:

■ *Disordered cerebral function* e.g. nausea, vomiting, headache, confusion and in severe cases convulsions, coma, and even death.

■ *Plasma changes* include reduced plasma electrolytes with decreased osmolality, lowered plasma proteins and reduced PCV.

DEHYDRATION

Dehydration or pure water deficiency is a state of deprivation of water without corresponding loss of electrolytes.

PATHOGENESIS. Decrease of body water initially affects intravascular compartment so that the blood volume is reduced. This is followed by withdrawing of fluid from the interstitial compartment with consequent hyperosmolality. This leads to shifting of intracellular water to the extracellular compartment which results in cellular dehydration as well as depletion of potassium ions from the cells. Release of ADH secretion in response to hypovolaemia results in reduced renal excretion of water. Sodium excretion is initially normal but later the renin-angiotensin-aldosterone mechanism is stimulated so that sodium is retained and its excretion decreases. Thus, this type of dehydration is characterised by water and potassium loss.

CAUSES. Pure water deficiency is less common than salt depletion but can occur in the following conditions:

1. Deficient water intake e.g. in dysphagia, obstructive lesion in oesophagus, starvation, intense weakness, coma.
2. Excessive loss of water e.g. in diabetes insipidus, hyperparathyroidism, pyrexia, hyperapnoea.

CLINICAL AND BIOCHEMICAL EFFECTS. These changes are as follows:

■ *Clinical effects* are intense thirst, mental confusion, fever, oliguria.

■ *Plasma changes.* In early stage, the levels of sodium, plasma proteins and PCV are unaltered but in late stage, there is rise in blood urea, serum sodium and PCV.

DISTURBANCES OF ELECTROLYTES

Changes in the concentration of sodium, potassium, chloride, bicarbonate, water and blood pH are briefly discussed below.

COMBINED SODIUM AND WATER DEFICIENCY

Combined sodium and water deficiency is more common than deficiency of either component separately. It is commonly called salt depletion because both sodium and chloride are lost equally.

PATHOGENESIS. Combined loss of water and sodium leads to fall in the volume of intravascular fluid first, and then reduction in extracellular fluid. Initially, there is no fall in the sodium level. But later, in response to ADH secretion, water is retained and sodium level falls. Thus, this type of dehydration is characterised by severe water and sodium loss.

CAUSES. Loss of sodium and water can occur in the following conditions:

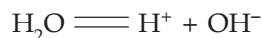
1. Gastrointestinal disorders e.g. severe vomitings, diarrhoea.
2. Excess loss via the sweat.
3. Excess loss via urine e.g. in patients of heart failure on diuretics, Addison's disease, advanced diabetes mellitus.
4. Excess loss via blood and plasma e.g. in severe haemorrhage, extensive burns.

CLINICAL AND BIOCHEMICAL EFFECTS. These are:

- *Clinical effects* are signs and symptoms of dehydration such as sunken eyeballs, wrinkled skin, dry tongue, muscle cramps, oliguria.
- *Plasma changes.* There is reduced level of plasma sodium, and raised levels of plasma proteins, PCV and blood urea.

ABNORMALITIES OF THE pH OF BLOOD

The pH may be defined as the negative logarithm of the H^+ ion concentration of a solution. In health, the pH of blood is kept constant at 7.4 ± 0.05 . In the body, pure water dissociates into very minute amounts of equal number of hydrogen ions and hydroxyl ions (10^{-7} each):



An increased concentration of H^+ ions shifts the pH lower (acidosis) whereas a decreased concentration of H^+ ions shifts the pH higher (alkalosis).

The pH of the blood is normally maintained according to Henderson-Hasselbalch equation:

$$pH = pK + \log \frac{\text{conjugate base } (HCO_3^-)}{\text{conjugate acid } (P_{CO_2})}$$

where pK is the dissociation constant of the carbonic acid which is 6.1, the concentration of the base (HCO_3^-) is 24 mmol/litre, and the concentration of the acid (carbonic acid) in solution at partial pressure of CO_2 (P_{CO_2}) of 40 mmHg (the normal alveolar tension) is 1.2 mmol/litre.

$$\text{Thus, pH of blood} = 6.1 + \log \frac{24}{1.2}$$

$$\begin{aligned} &= 6.1 + \log 20 \\ &= 6.1 + 1.3 \\ &= 7.4 \end{aligned}$$

Therefore, the pH of blood depends upon 2 principal factors:

- the *concentration of bicarbonate*; and
- the *partial pressure of CO_2* that determines the concentration of carbonic acid.

Accordingly, the disorders of the pH of the blood can be of 2 types:

1. *alterations in the blood bicarbonate levels* (metabolic acidosis and alkalosis); and
2. *alteration in P_{CO_2}* which depends upon the ventilatory function of the lungs (respiratory acidosis and alkalosis).

Metabolic Acidosis

A fall in the blood pH due to metabolic component is brought about by fall of bicarbonate level and excess of H^+ ions in the blood. This occurs in the following situations:

- Production of large amounts of lactic acid (lactic acidosis) e.g. in vigorous exercise, shock.
- Uncontrolled diabetes mellitus (diabetic keto-acidosis).
- Starvation.
- Chronic renal failure.
- Therapeutic administration of ammonium chloride or acetazolamide (diamox).

High blood levels of H^+ ions in metabolic acidosis stimulate the respiratory centre so that the breathing is deep and rapid (*air hunger* or *Kussmaul's respiration*). There is fall in the plasma bicarbonate levels.

Metabolic Alkalosis

A rise in the blood pH due to rise in the bicarbonate levels of plasma and loss of H^+ ions is called metabolic alkalosis. This is seen in the following conditions:

- Severe and prolonged vomitings.
- Administration of alkaline salts like sodium bicarbonate.
- Hypokalaemia such as in Cushing's syndrome, increased secretion of aldosterone.

Clinically, metabolic alkalosis is characterised by depression of respiration, depressed renal function with uraemia and increased bicarbonate excretion in the urine. The blood level of bicarbonate is elevated.

Respiratory Acidosis

A fall in the blood pH occurring due to raised P_{CO_2} consequent to underventilation of lungs (CO_2 retention) causes respiratory acidosis. This can occur in the following circumstances:

- Air obstruction as occurs in chronic bronchitis, emphysema, asthma.
- Restricted thoracic movement e.g. in pleural effusion, ascites, pregnancy, kyphoscoliosis.
- Impaired neuromuscular function e.g. in poliomyelitis, polyneuritis.

Clinically, there is peripheral vasodilatation and raised intracranial pressure. If there is severe CO_2 retention, patients may develop confusion, drowsiness and coma. The arterial Pco_2 level is raised.

Respiratory Alkalosis

A rise in the blood pH occurring due to lowered Pco_2 consequent to hyperventilation of the lungs (excess removal of CO_2) is called respiratory alkalosis. This is encountered in the following conditions:

- Hysterical overbreathing
- Working at high temperature
- At high altitude
- Meningitis, encephalitis
- Salicylate intoxication

Clinically, the patients with respiratory alkalosis are characterised by peripheral vasoconstriction and consequent pallor, lightheadedness and tetany. The arterial Pco_2 is lowered.



Circulatory Disturbances Due to Deranged Volume

There are three main basic requirements for normal circulatory function: normal anatomic features, normal physiologic controls, and normal biochemical composition of the blood. These are essential to maintain normal blood flow and perfusion of tissues.

Derangements of blood flow or haemodynamic disturbances are considered under 2 broad headings:

- I. *Disturbances in the volume of the circulating blood.* These include: hyperaemia and congestion, haemorrhage and shock.
- II. *Circulatory disturbances of obstructive nature.* These are: thrombosis, embolism (Chapter 17), ischaemia and infarction (Chapter 18).

HYPERAEMIA AND CONGESTION

Hyperaemia and congestion are the terms used for increased volume of blood within dilated vessels of an organ or tissue; the increased volume from arterial and arteriolar dilatation being referred to as *hyperaemia* or *active hyperaemia*, whereas the impaired venous drainage is called *venous congestion* or *passive hyperaemia*. If the condition develops rapidly it is called *acute*, while more prolonged and gradual response is known as *chronic*.

Active Hyperaemia

The dilatation of arteries, arterioles and capillaries is effected either through sympathetic neurogenic mechanism or via the release of vasoactive substances. The affected tissue or organ is pink or red in appearance (erythema).

The *examples* of active hyperaemia are seen in the following conditions:

- Inflammation e.g. congested vessels in the walls of alveoli in pneumonia.
- Blushing i.e. flushing of the skin of face in response to emotions.
- Menopausal flush.
- Muscular exercise.
- High grade fever.

Passive Hyperaemia (Venous Congestion)

The dilatation of veins and capillaries due to impaired venous drainage results in passive hyperaemia or venous

congestion, commonly referred to as *congestion*. Congestion may be acute or chronic, the latter being more common and called *chronic venous congestion* (CVC). The affected tissue or organ is bluish in colour due to accumulation of venous blood (cyanosis). Obstruction to the venous outflow may be local or systemic. Accordingly, venous congestion is of 2 types:

■ *Local venous congestion* results from obstruction to the venous outflow from an organ or part of the body e.g. portal venous obstruction in cirrhosis of the liver, outside pressure on the vessel wall as occurs in tight bandage, plasters, tumours, pregnancy, hernia etc, or intraluminal occlusion by thrombosis.

■ *Systemic (General) venous congestion* is engorgement of systemic veins e.g. in left-sided and right-sided heart failure and diseases of the lungs which interfere with pulmonary blood flow like pulmonary fibrosis, emphysema etc. Usually the fluid accumulates upstream to the specific chamber of the heart which is initially affected. For example, in *left-sided heart failure* (such as due to mechanical overload in aortic stenosis, or due to weakened left ventricular wall as in myocardial infarction) pulmonary congestion results, whereas in *right-sided heart failure* (such as due to pulmonary stenosis or pulmonary hypertension) systemic venous congestion results. Fig. 16.1 illustrates the mechanisms involved in passive or venous congestion of different organs.

MORPHOLOGY OF CVC OF ORGANS

Morphologic changes seen in CVC of the lungs, liver, spleen and kidney are discussed below.

CVC Lung

Chronic venous congestion of lung occurs in left heart failure, especially in rheumatic mitral stenosis so that there is consequent rise in pulmonary venous pressure.

Grossly, the lungs are heavy and firm in consistency. The sectioned surface is dark brown in colour referred to as *brown induration* of the lungs.

Microscopically, the alveolar septa are widened due to the presence of interstitial oedema as well as due

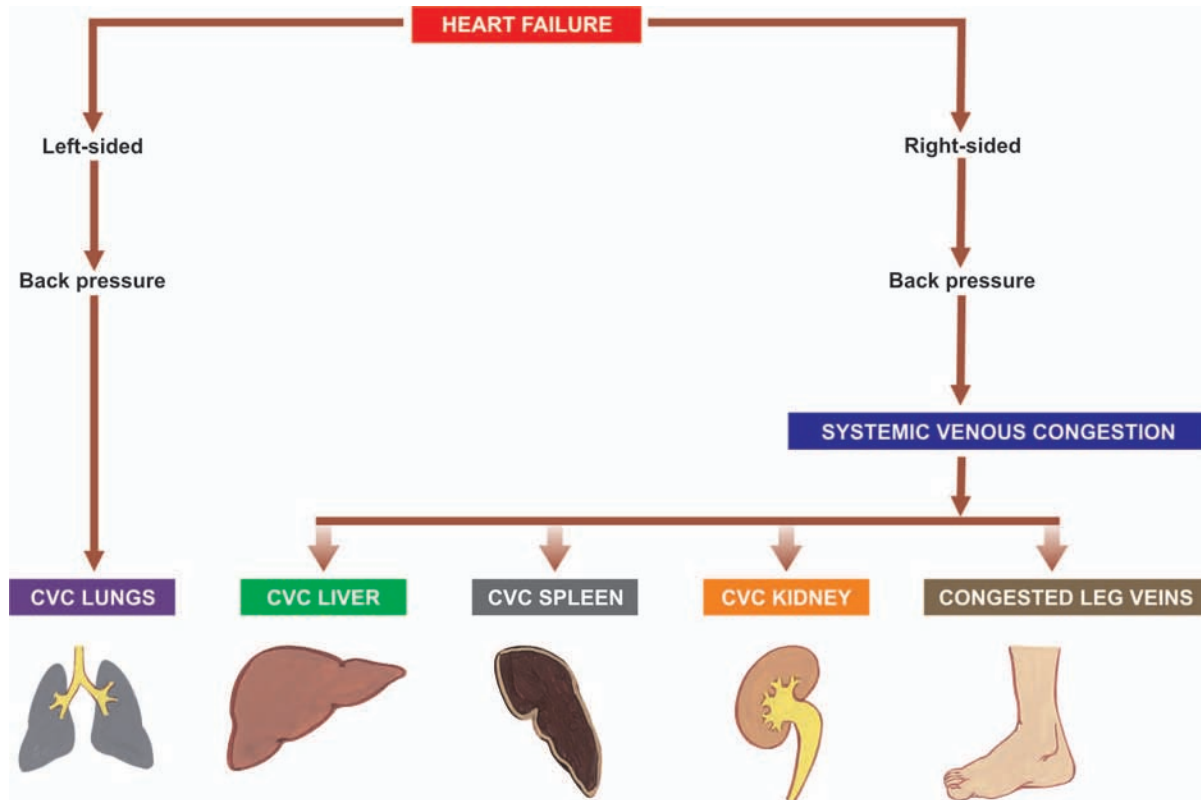


FIGURE 16.1

Schematic representation of mechanisms involved in chronic venous congestion (CVC) of different organs.

to dilated and congested capillaries. The septa are mildly thickened due to slight increase in fibrous connective tissue. Rupture of dilated and congested capillaries may result in minute intra-alveolar haemorrhages. The breakdown of erythrocytes liberates haemosiderin pigment which is taken up by alveolar macrophages, so called *heart failure cells*, present in the alveolar lumina. The brown induration of the cut surface of the lungs is due to the pigmentation and fibrosis (Fig. 16.2).

CVC Liver

Chronic venous congestion of the liver occurs in right heart failure and sometimes due to occlusion of inferior vena cava and hepatic vein.

Grossly, the liver is enlarged and tender and the capsule is tense. Cut surface shows characteristic *nutmeg* liver due to red and yellow mottled appearance, corresponding to congested centre of lobules and fatty peripheral zone respectively (Fig. 16.3).

Microscopically, the changes of congestion are more marked in the centrilobular zone due to severe hypoxia than in the peripheral zone. The central veins as well as the adjacent sinusoids are distended and filled with blood. The centrilobular hepatocytes undergo degenerative changes, and eventually *centrilobular haemorrhagic necrosis* may be seen. Long-standing cases may show fine centrilobular fibrosis and regeneration of hepatocytes, resulting in cardiac cirrhosis. The peripheral zone of the lobule is less severely affected by chronic hypoxia and shows some *fatty change* in the hepatocytes (Fig. 16.4) (COLOUR PLATE III: CL 10).

CVC Spleen

Chronic venous congestion of the spleen occurs in right heart failure and in portal hypertension from cirrhosis of liver.

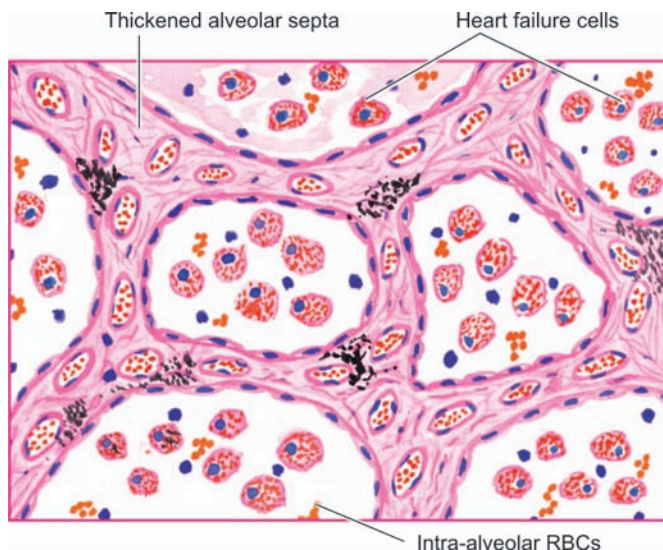


FIGURE 16.2

Histological appearance of CVC lung. The alveolar walls are widened and thickened due to congestion, oedema and mild fibrosis and the alveolar lumina contain heart failure cells.

Grossly, the spleen in early stage is slightly to moderately enlarged (upto 250 g as compared to normal 150 g), while in long-standing cases there is progressive enlargement and may weigh upto 500 to 1000 g. The organ is deeply congested, tense and cyanotic. Sectioned surface is gray tan.

Microscopically, the red pulp shows congestion and marked sinusoidal dilatation with areas of recent and old haemorrhages. These haemorrhages may get organised and form Gamna-Gandy bodies or sidero-

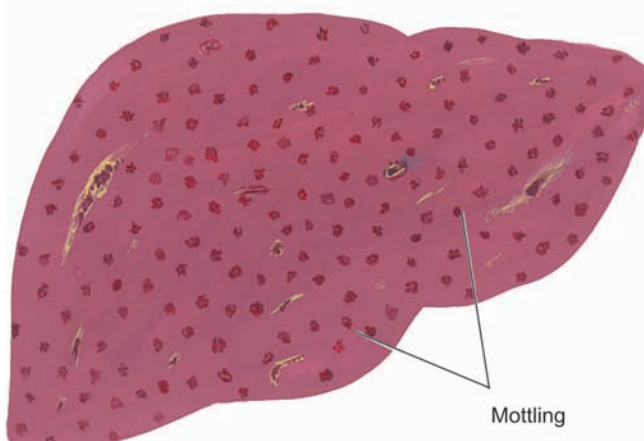


FIGURE 16.3

Nutmeg liver. The cut surface shows mottled appearance—alternate pattern of dark congestion and pale fatty change.

fibrotic nodules which are deposits of haemosiderin pigment and calcium salts on fibrous connective tissue and elastic fibres. The reticulin-network as well as fibrous trabeculae are thickened (Fig. 16.5). In the late stage, there is hyperplasia of macrophages and fibroblasts resulting in increase in fibrous tissue of the capsule and hyperplasia of red pulp all of which account for firmness of the spleen (**COLOUR PLATE III: CL 9**). This advanced stage seen more commonly in hepatic cirrhosis is called *congestive splenomegaly* and is the commonest cause of hypersplenism (page 538).

CVC Kidney

Grossly, the kidneys are slightly enlarged and the medulla is congested.

Microscopically, the changes are rather mild. The tubules may show degenerative changes like cloudy swelling and fatty change. The glomeruli may show mesangial proliferation.

HAEMORRHAGE

Haemorrhage is the escape of blood from a blood vessel. The bleeding may occur *externally*, or *internally* into the serous cavities (e.g. haemothorax, haemoperitoneum, haemopericardium) or into a hollow viscus. Extravasation of blood into the tissues with resultant swelling is

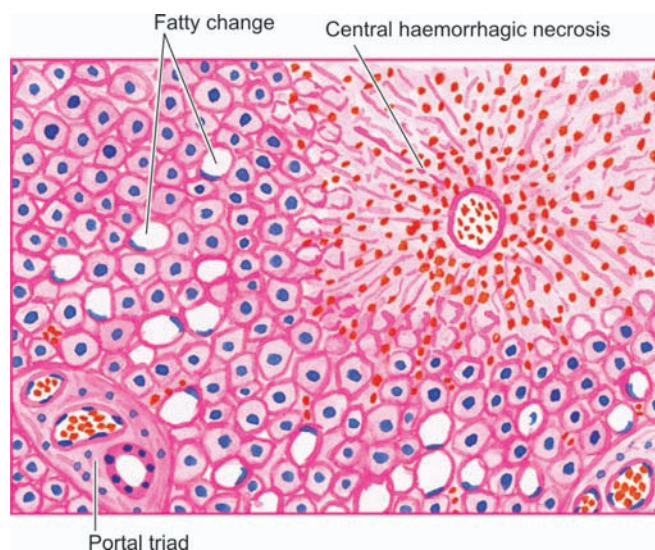


FIGURE 16.4

CVC liver. The centrilobular zone shows marked degeneration and necrosis of hepatocytes accompanied by haemorrhage while the peripheral zone shows mild fatty change of liver cells.

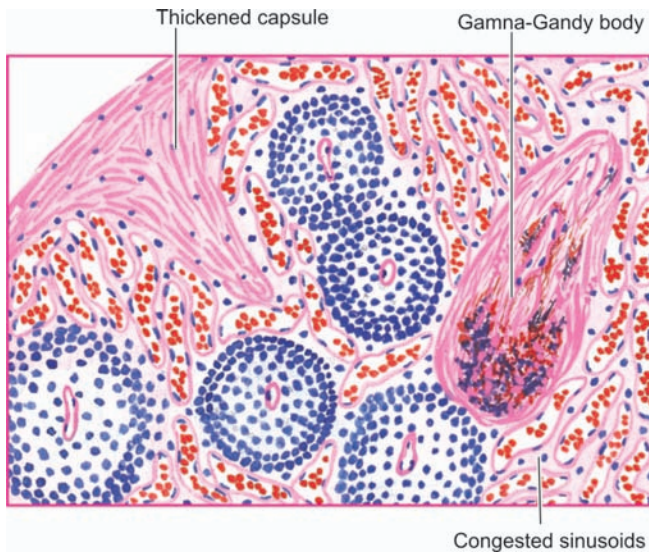


FIGURE 16.5

Histological appearance of CVC spleen. The sinusoids are dilated and congested. There is increased fibrosis in the red pulp, capsule and the trabeculae. Gamna-Gandy body is also seen.

known as *haematoma*. Large extravasations of blood into the skin and mucous membranes are called *ecchymoses*. *Purpuras* are small areas of haemorrhages (upto 1 cm) into the skin and mucous membrane, whereas *petechiae* are minute pinhead-sized haemorrhages. Microscopic escape of erythrocytes into loose tissues may occur following marked congestion and is known as *diapedesis*.

CAUSES OF HAEMORRHAGE. The blood loss may be large and sudden (*acute*), or small repeated bleeds may occur over a period of time (*chronic*). The various causes of haemorrhage are listed below:

1. *Trauma* to the vessel wall e.g. penetrating wound in the heart or great vessels, during labour etc.
2. *Spontaneous haemorrhage* e.g. rupture of an aneurysm, septicaemia, bleeding diathesis (such as purpura), acute leukaemias, pernicious anaemia, scurvy.
3. *Inflammatory lesions of the vessel wall* e.g. bleeding from chronic peptic ulcer, typhoid ulcers, blood vessels traversing a tuberculous cavity in the lung, syphilitic involvement of the aorta, polyarteritis nodosa.
4. *Neoplastic invasion* e.g. haemorrhage following vascular invasion in carcinoma of the tongue.
5. *Vascular diseases* e.g. atherosclerosis.
6. *Elevated pressure within the vessels* e.g. cerebral and retinal haemorrhage in systemic hypertension, severe haemorrhage from varicose veins due to high pressure in the veins of legs or oesophagus.

EFFECTS OF HAEMORRHAGE. The effects of blood loss depend upon 3 main factors:

- the amount of blood loss;
- the speed of blood loss; and
- the site of the haemorrhage.

The loss upto 20% of blood volume suddenly or slowly generally has little clinical effects because of compensatory mechanisms. A sudden loss of 33% of blood volume may cause death, while loss of upto 50% of blood volume over a period of 24 hours may not be necessarily fatal. However, chronic blood loss generally produces an iron deficiency anaemia, whereas acute haemorrhage may lead to serious immediate consequences such as hypovolaemic shock.

SHOCK

Definition and Types

Shock is defined as a clinical state of cardiovascular collapse characterised by:

- an acute reduction of effective circulating blood volume; and
- an inadequate perfusion of cells and tissues.

The end result is hypotension and cellular hypoxia and, if uncompensated, may lead to impaired cellular metabolism and death. Shock may be of 2 main types: primary (initial) and secondary (true) shock.

PRIMARY OR INITIAL SHOCK. It is transient and usually a benign vasovagal attack resulting from sudden reduction of venous return to the heart caused by neurogenic vasodilatation and consequent peripheral pooling of blood. It can occur immediately following trauma, severe pain or emotional overreaction such as due to fear, sorrow or surprise. Clinically, the patient generally develops unconsciousness, weakness, sinking sensation, pale and clammy limbs, weak and rapid pulse, and low blood pressure. The attack usually lasts for a few seconds or minutes.

SECONDARY OR TRUE SHOCK. This is the form of shock which occurs due to haemodynamic derangements with hypoperfusion of the cells. This type of shock is the true shock which is commonly referred to as 'shock' if not specified and is the type described below.

Etiology and Classification

Many types of injuries and diseases can cause shock. These causes are broadly grouped under 3 major headings, and accordingly shock is classified into 3 main etiologic forms: hypovolaemic, cardiogenic, and septic.

1. HYPOVOLAEMIC SHOCK. Reduction in blood volume induces hypovolaemic shock. The causes of hypovolaemia include the following:

- i) *Severe haemorrhage* (external or internal) e.g. in trauma, surgery.
- ii) *Fluid loss* e.g. in severe burns, crush injury to a limb, persistent vomitings and severe diarrhoea causing dehydration.

2. SEPTIC SHOCK. Severe bacterial infections or septicaemia induce septic shock. The predominant causes are as under:

i) **Gram-negative septicaemia (endotoxic shock)** e.g. infection with *E. coli*, *Proteus*, *Klebsiella*, *Pseudomonas* and bacteroides. Endotoxins of gram-negative bacilli have been implicated as the most important mediator of septic shock.

ii) **Gram-positive septicaemia (exotoxic shock)** is less common e.g. infection with streptococci, pneumococci.

As shown schematically in Fig. 16.6, lysis of gram-negative bacteria releases endotoxin, a lipopolysaccharide (LPS), into circulation where it binds to lipopolysaccharide-binding protein (LBP). The complex of LPS-LBP binds to CD14 molecule on the surface of the monocyte/macrophage which in turn is stimulated to elaborate tumour necrosis factor- α (TNF- α). TNF- α induces septic shock by endothelial cell injury by many mechanisms which include:

- a) Direct cytotoxicity
- b) Promotes the adherence of polymorphs to endothelium
- c) Stimulates the release of interleukin-1 (IL-1)
- d) Promotes the release of procoagulant tissue factor that causes thrombosis and local ischaemia.

3. CARDIOGENIC SHOCK. Acute circulatory failure with sudden fall in cardiac output from acute diseases of the heart without actual reduction of blood volume (normovolaemia) results in cardiogenic shock. The causes include the following:

- i) *Deficient emptying* e.g.
 - Myocardial infarction
 - Rupture of the heart
 - Cardiac arrhythmias
- ii) *Deficient filling* e.g.
 - Cardiac tamponade from haemopericardium
- iii) *Obstruction to the outflow* e.g.
 - Pulmonary embolism
 - Ball valve thrombus.

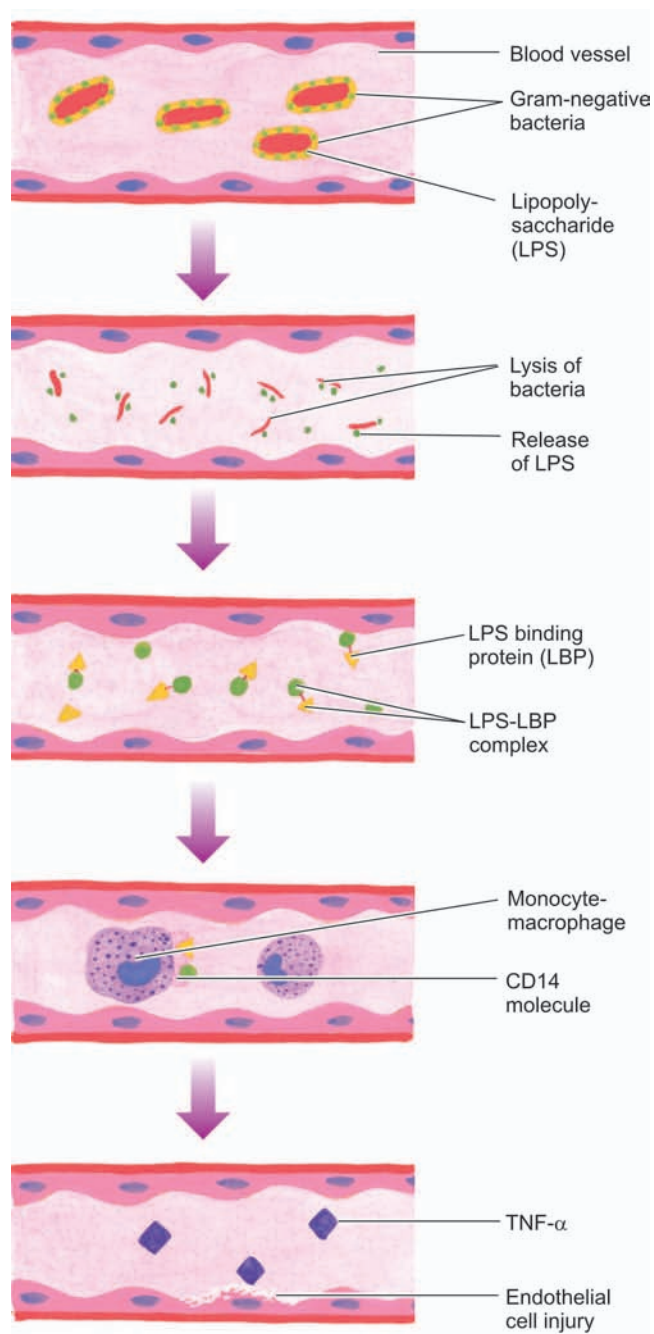


FIGURE 16.6

Pathogenesis of endotoxic shock.

Besides the three major forms of shock described above, *neurogenic shock* (following anaesthesia or spinal cord injury) and *anaphylactic shock* are two other types of shock resulting from peripheral vasodilation with pooling of blood.

Pathogenesis

There are 2 basic features in the pathogenesis of shock:

- reduced effective circulating blood volume; and
- reduced supply of oxygen to the cells and tissues with resultant anoxia.

1. REDUCED EFFECTIVE CIRCULATING VOLUME.

It may result by either of the following mechanisms:

- i) by actual loss of blood volume as occurs in hypovolaemic shock; or
- ii) by decreased cardiac output without actual loss of blood (normovolaemia) as occurs in cardiogenic shock and septic shock.

2. TISSUE ANOXIA. Following reduction in the effective circulating blood volume from either of the above two mechanisms and from any of the etiologic agents, there is decreased venous return to the heart resulting in decreased cardiac output. This consequently causes reduced supply of oxygen to the organs and tissues and hence tissue anoxia, and shock ensues.

In contrast to hypovolaemic and cardiogenic shock, patients in septic shock have hyperdynamic circulation

due to peripheral vasodilatation and pooling of blood, as well as there is increased vascular permeability. This results in reduction of effective circulating blood volume, lowered cardiac output, reduced blood flow (hypotension) and inadequate perfusion of cells and tissues. Disseminated intravascular coagulation (DIC) is prone to develop in septic shock due to endothelial cell injury by toxins.

The sequence of events in the pathogenesis of shock is summarised in Fig. 16.7.

Stages of Shock

Deterioration of the circulation in shock is a progressive phenomenon and can be divided arbitrarily into 3 stages:

1. Non-progressive (initial, compensated reversible) shock.
2. Progressive decompensated shock.
3. Decompensated (irreversible) shock.

1. NON-PROGRESSIVE (INITIAL, COMPENSATED REVERSIBLE) SHOCK. In the early stage of shock, an

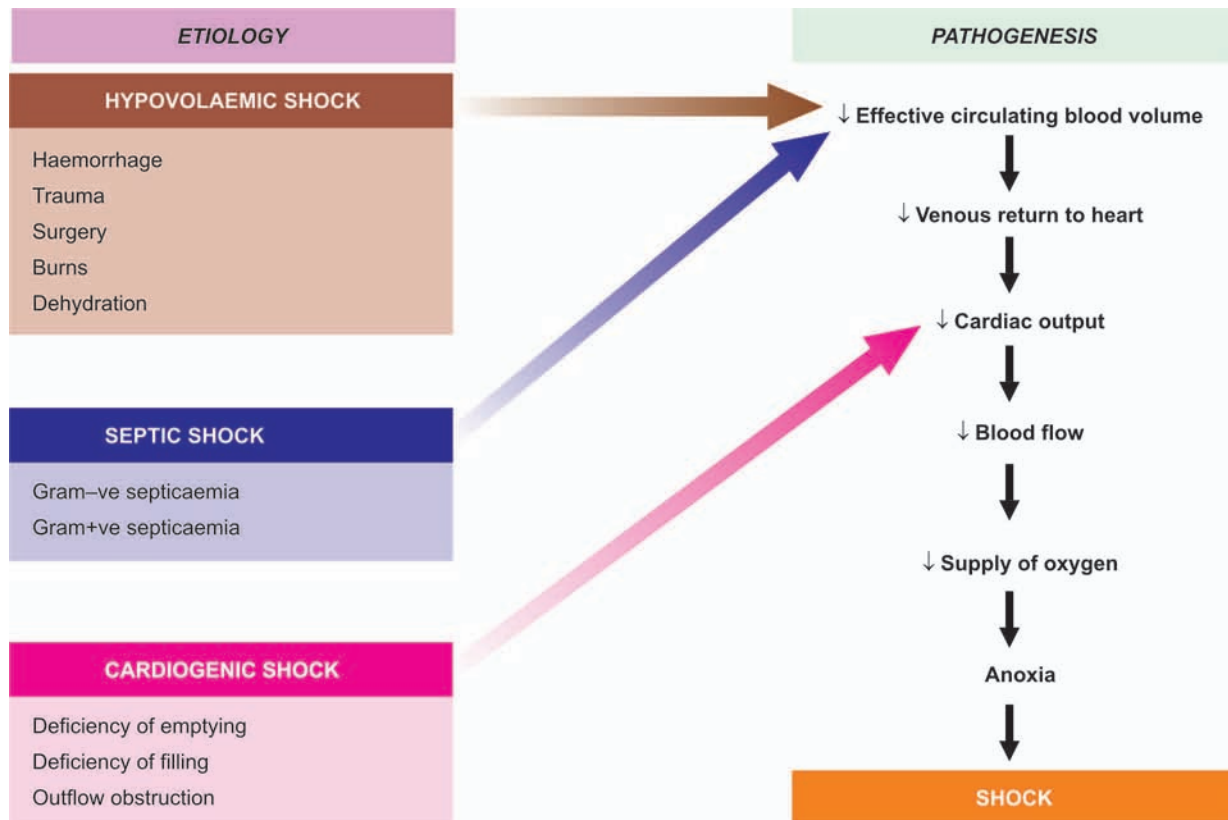


FIGURE 16.7

Etiology (left) and pathogenesis (right) of shock.

attempt is made to maintain adequate cerebral and coronary blood supply by redistribution of blood so that the vital organs (brain and heart) are adequately perfused and oxygenated. This is achieved by activation of various neurohormonal mechanisms causing *widespread vasoconstriction* and by *fluid conservation by the kidney*. If the condition that caused the shock is adequately treated, the compensatory mechanism may be able to bring about recovery and re-establish the normal circulation; this is called compensated or reversible shock. These compensatory mechanisms are as under:

i) Widespread vasoconstriction. In response to reduced blood flow (hypotension) and tissue anoxia, the neural and humoral factors (e.g. baroreceptors, chemoreceptors, catecholamines, renin, and VEM or vasoexcitator material from hypoxic kidney) are activated. All these bring about vasoconstriction, particularly in the vessels of the skin and abdominal viscera. Widespread vasoconstriction is a protective mechanism as it causes increased peripheral resistance, increased heart rate (tachycardia) and increased blood pressure. However, in septic shock, there is initial vasodilatation followed by vasoconstriction. Besides, in severe septic shock there is elevated level of thromboxane A₂ which is a potent vasoconstrictor and may augment the cardiac output alongwith other sympathetic mechanisms. Clinically cutaneous vasoconstriction is responsible for cool and pale skin in initial stage of shock.

ii) Fluid conservation by the kidney. In order to compensate the actual loss of blood volume in hypovolaemic shock, the following factors may assist in restoring the blood volume and improve venous return to the heart:

- Release of aldosterone from hypoxic kidney.
- Release of ADH due to decreased effective circulating blood volume.
- Reduced glomerular filtration rate (GFR) due to arteriolar constriction.
- Shifting of tissue fluids into the plasma due to lowered capillary hydrostatic pressure (hypotension).

iii) Vascular autoregulation. In response to hypoxia and acidosis, regional blood flow to the heart and brain is preserved by vasodilatation of the coronary and cerebral circulation.

2. PROGRESSIVE DECOMPENSATED SHOCK. This is a stage when the patient suffers from some other stress or risk factors (e.g. pre-existing cardiovascular and lung disease) besides persistence of the shock so that there is progressive deterioration. The effects of progressive decompensated shock due to tissue hypoperfusion are as under:

- a) Pulmonary hypoperfusion with resultant tachypnoea and adult respiratory distress syndrome.
- b) Tissue anoxia causing anaerobic glycolysis results in metabolic lactic acidosis. Lactic acidosis lowers the tissue pH which in turn makes the vasomotor response ineffective. This results in vasodilation and peripheral pooling of blood.

Clinically at this stage the patient develops confusion and worsening of renal function.

3. DECOMPENSATED (IRREVERSIBLE) SHOCK.

When the shock is so severe that in spite of compensatory mechanisms and despite therapy and control of etiologic agent which caused the shock, no recovery takes place, it is called decompensated or irreversible shock. Its effects due to widespread cell injury include the following:

- a) Progressive fall in the blood pressure due to deterioration in cardiac output attributed to release of myocardial depressant factor (MDF).
- b) Severe metabolic acidosis due to anaerobic glycolysis.
- c) Respiratory distress due to pulmonary oedema, tachypnoea and adult respiratory distress syndrome (ARDS).
- d) Ischaemic cell death of brain, heart and kidneys due to reduced blood supply to these organs.

Clinically, at this stage the patient has features of coma, worsened heart function and progressive renal failure due to acute tubular necrosis.

A number of hypotheses or factors have been described in irreversibility of shock, with tissue anoxia occupying the central role (Fig.16.8). These are as under:

i) Persistence of widespread vasoconstriction. Although widespread vasoconstriction is a compensatory mechanism, its persistence for prolonged duration can cause anoxia of tissues and organs like the liver, spleen, kidney and intestine. Particularly significant is the postcapillary venular constriction contributing to tissue anoxia.

ii) Vasodilatation and increased vascular permeability. Anoxia damages the capillary and venular wall so that there is vasodilatation and increased vascular permeability. Vasodilatation results in peripheral pooling of blood while increased vascular permeability causes escape of fluid from circulation into the interstitial tissues, both of which further deteriorate the effective circulating blood volume.

iii) Myocardial ischaemia. Persistently reduced blood flow to myocardium causes coronary insufficiency and

STAGE	PATHOGENESIS	EFFECTS
INITIAL SHOCK	• Baroreceptor • Catecholamine release • Renin-angiotensin activation • ADH release • Sympathetic stimulation	• Peripheral vasoconstriction • Cool clammy skin • Tachycardia • Fluid conservation by kidney
PROGRESSIVE SHOCK	• ARDS • Anaerobic glycolysis • Lactic acidosis, Low tissue pH • Impaired vasomotor response	• ↓ Cardiac output • DIC • Mental confusion • ↓ Urinary output
IRREVERSIBLE SHOCK	• Persistent vasoconstriction • Vasodilatation & vascular permeability • Myocardial ischaemia (MDF) • Cerebral ischaemia • VDM • TNF • Intestinal factor • Bacterial factor • Hypercoagulability	• Brain: death • Lungs: ARDS • Heart: focal myocardial necrosis • Kidney: ATN • Liver: necrosis • Spleen: hyperplasia • Stomach: ulcer • Intestine: necrosis • Splanchnic: vasodilatation

FIGURE 16.8

Mechanisms and effects of three stages of shock.

myocardial ischaemia due to release of myocardial depressant factor (MDF). This results in depression of cardiac function, reduced cardiac output and decreased blood flow.

iv) Cerebral ischaemia. Cerebral ischaemia resulting from persistent reduction of blood flow causes depression of the vasomotor centre. This results in vasodilatation and peripheral pooling of blood, thus reducing the venous return to the heart and consequent lowered cardiac output.

v) Vasodepressor material (VDM). VDM is a substance produced by the spleen and skeletal muscle and is normally inactivated in the liver. In severe hypoxia of the liver as occurs in irreversible shock, the mechanism of inactivation of VDM in liver is damaged so that its blood levels rise. VDM causes peripheral vasodilatation (reverse of VEM) and thus diverts blood from the systemic circulation, resulting in deterioration of the circulation.

vi) Tumour necrosis factor (TNF). In septic shock, monocyte-macrophage cell system gets activated by bacterial products causing release of substances like prostaglandins, leukotrienes, platelet activating factor and interleukins-1, 6 and 18. These substances have been implicated in producing irreversibility of endotoxic shock.

vii) Intestinal factor. Due to prolonged vasoconstriction, haemorrhagic necrosis of the intestinal tract occurs. This results in loss of blood and plasma into the intestine from haemorrhagic lesions, causing further reduction in effective circulating blood volume.

viii) Bacterial factor. Prolonged anoxic injury to the reticuloendothelial organs like the liver and spleen impairs the normal anti-bacterial defense mechanism of these organs. This results in release of undetoxified endotoxins derived from intestinal bacteria into the circulation, which cause further vasoconstriction and its harmful effects.

ix) Hypercoagulability of blood. Excessive accumulation of lactic acid in the blood in prolonged shock enhances the release of catecholamines (e.g. epinephrine) into the circulation. The effects of catecholamines include the release of clot promoting factor, release of thromboplastin and release of platelet aggregator, ADP. Excess lactic acid in the blood can also cause endothelial injury and thus initiate thrombosis. In this way, hypercoagulability of blood with consequent microthrombi impair the blood flow and may even cause tissue necrosis.

Morphologic Complications in Shock

Eventually, shock is characterised by multisystem failure. The morphologic changes in shock are due to

hypoxia resulting in degeneration and necrosis in various organs. The major organs affected are the brain, heart, lungs and kidneys. Morphologic changes are also noted in the adrenals, gastrointestinal tract, liver and other organs. The predominant morphologic complications of shock are shown in Fig. 16.8 and described below.

1. HYPOXIC ENCEPHALOPATHY. Cerebral ischaemia in compensated shock may produce altered state of consciousness. However, if the blood pressure falls below 50 mmHg as occurs in systemic hypotension in prolonged shock and cardiac arrest, brain suffers from serious ischaemic damage with loss of cortical functions, coma, and a vegetative state.

Grossly, the area supplied by the most distal branches of the cerebral arteries suffers from severe ischaemic necrosis which is usually the border zone between the anterior and middle cerebral arteries.

Microscopically, the changes are noticeable if ischaemia is prolonged for 12 to 24 hours. Neurons, particularly Purkinje cells, are more prone to develop the effects of ischaemia. The cytoplasm of the affected neurons is intensely eosinophilic and the nucleus is small pyknotic. Dead and dying nerve cells are replaced by gliosis.

2. HEART IN SHOCK. Heart is more vulnerable to the effects of hypoxia than any other organ. Heart is affected in cardiogenic as well as in other forms of shock. There are 2 types of morphologic changes in heart in all types of shock:

- i) *Haemorrhages and necrosis.* There may be small or large ischaemic areas or infarcts, particularly located in the subepicardial and subendocardial region.
- ii) *Zonal lesions.* These are opaque transverse contraction bands in the myocytes near the intercalated disc.

3. SHOCK LUNG. Lungs due to dual blood supply are generally not affected by hypovolaemic shock but in septic shock the morphologic changes in lungs are quite prominent termed 'shock lung'.

Grossly, the lungs are heavy and wet.

Microscopically, changes of adult respiratory distress syndrome (ARDS) are seen. Briefly, the changes include congestion, interstitial and alveolar oedema, interstitial lymphocytic infiltrate, alveolar hyaline membranes, thickening and fibrosis of alveolar septa, and fibrin and platelet thrombi in the pulmonary microvasculature.

4. SHOCK KIDNEY. One of the important complications of shock is irreversible renal injury, first noted in persons who sustained crush injuries in building collapses in air raids in World War II. The renal ischaemia following systemic hypotension is considered responsible for renal changes in shock. The end-result is generally anuria and death.

Grossly, the kidneys are soft and swollen. Sectioned surface shows blurred architectural markings.

Microscopically, the tubular lesions are seen at all levels of nephron and are referred to as acute tubular necrosis (ATN) which can occur following other causes besides shock. If extensive muscle injury or intravascular haemolysis are also associated, peculiar brown tubular casts are seen.

5. ADRENALS IN SHOCK. The adrenals show stress response in shock. This includes release of aldosterone in response to hypoxic kidney, release of glucocorticoids from adrenal cortex and catecholamines like adrenaline from adrenal medulla. In severe shock, adrenal haemorrhages may occur.

6. HAEMORRHAGIC GASTROENTEROPATHY. The hypoperfusion of the alimentary tract in conditions such as shock and cardiac failure may result in mucosal and mural infarction called haemorrhagic gastroenteropathy. This type of non-occlusive ischaemic injury of bowel must be distinguished from full-fledged infarction in which case the deeper layers of gut (muscularis and serosa) are also damaged. In shock due to burns, acute stress ulcers of the stomach or duodenum may occur and are known as Curling's ulcers.

Grossly, the lesions are multifocal and widely distributed throughout the bowel. The lesions are superficial ulcers, reddish purple in colour. The adjoining bowel mucosa is oedematous and haemorrhagic.

Microscopically, the involved areas show dilated and congested vessels and haemorrhagic necrosis of the mucosa and sometimes submucosa. Secondary infection may supervene and condition may progress into pseudomembranous enterocolitis.

7. LIVER IN SHOCK. Due to effects of hypoxia on liver, VDM is released from the liver which causes vasodilatation. Besides, focal necrosis may be seen, fatty change may occur and the liver function may be impaired.

8. OTHER ORGANS. Other organs such as lymph nodes, spleen and pancreas may also show foci of necrosis in shock. In addition, the patients who survive

acute phase of shock succumb to overwhelming infection due to altered immune status and host defense mechanism.

Clinical Features

The classical features of decompensated shock are characterised by *depression of 4 vital processes*:

- Very low blood pressure
- Subnormal temperature
- Feeble and irregular pulse
- Shallow and sighing respiration

In addition, the patients in shock have pale face, sunken eyes, weakness, cold and clammy skin. Renal dysfunction in shock is clinically characterised by a phase of oliguria due to ATN and a later phase of diuresis due to regeneration of tubular epithelium. Haemoconcentration is present in early oliguric phase while marked electrolyte imbalance occurs in diuretic phase. With progression of the condition, the patient may develop stupor, coma and death.



Circulatory Disturbances of Obstructive Nature

The second group of circulatory disturbances are obstructive in nature and include: thrombosis and embolism (discussed below), and ischaemia and infarction (discussed in the next chapter).

THROMBOSIS

Definition and Effects

Thrombosis is the process of formation of solid mass in circulation from the constituents of flowing blood; the mass itself is called a *thrombus*. A *blood clot* is the mass of coagulated blood formed *in vitro* e.g. in a test tube. *Haematoma* is the extravascular accumulation of blood clot e.g. into the tissues. *Haemostatic plugs* are the blood clots formed in healthy individuals at the site of bleeding e.g. in injury to the blood vessel. In other words, haemostatic plug at the cut end of a blood vessel may be considered the simplest form of thrombosis. Haemostatic plugs are useful as they stop the escape of blood and plasma, whereas thrombi developing in the unruptured cardiovascular system may be life-threatening by causing one of the following harmful effects:

1. **Ischaemic injury.** Thrombi may decrease or stop the blood supply to part of an organ or tissue and cause ischaemia which may subsequently result in infarction.
2. **Thromboembolism.** The thrombus or its part may get dislodged and be carried along in the bloodstream as embolus to lodge in a distant vessel.

Pathophysiology

Since the protective haemostatic plug formed as a result of normal haemostasis is an example of thrombosis, it is essential to describe *thrombogenesis* in relation to the normal haemostatic mechanism.

Human beings possess inbuilt system by which the blood remains in fluid state normally and guards against the hazards of thrombosis and haemorrhage. However, injury to the blood vessel initiates haemostatic repair mechanism or thrombogenesis. Virchow described three primary events which predispose to thrombus formation (*Virchow's triad*): endothelial injury, alteration in flow of blood, and hypercoagulability of blood. These events are discussed below:

1. ROLE OF BLOOD VESSEL WALL. The integrity of blood vessel wall is important for maintaining normal blood flow. An intact endothelium has the following functions:

- i) It *protects* the flowing blood from the thrombogenic influence of subendothelium.
- ii) It elaborates a few *anti-thrombotic* factors (thrombosis inhibitory factors) e.g.
 - a) Heparin-like substance which accelerates the action of antithrombin III and inactivates some other clotting factors.
 - b) Thrombomodulin which converts thrombin into activator of protein C, an anticoagulant.
 - c) Inhibitors of platelet aggregation such as ADPase, PGI₂ or prostacyclin.
 - d) Tissue plasminogen activator which accelerates the fibrinolytic activity.
- iii) It can release a few *prothrombotic factors* which have procoagulant properties (thrombosis favouring factors) e.g.
 - a) Thromboplastin or tissue factor released from endothelial cells.
 - b) von Willebrand factor that causes adherence of platelets to the subendothelium.
 - c) Platelet activating factor which is activator and aggregator of platelets.
 - d) Inhibitor of plasminogen activator that suppresses fibrinolysis.

Vascular injury exposes the subendothelial connective tissue (e.g. collagen, elastin, fibronectin, laminin and glycosaminoglycans) which are thrombogenic and thus plays important role in initiating haemostasis as well as thrombosis. Injury to vessel wall also causes vasoconstriction of small blood vessels briefly so as to reduce the blood loss. Endothelial injury is of major significance in the formation of arterial thrombi and thrombi of the heart, especially of the left ventricle. A number of factors and conditions may cause vascular injury and predispose to the formation of thrombi. These are as under:

- i) Endocardial injury in myocardial infarction, myocarditis, cardiac surgery, prosthetic valves.

- ii) Ulcerated plaques in advanced atherosclerosis.
- iii) Haemodynamic stress in hypertension.
- iv) Arterial diseases.
- v) Diabetes mellitus.
- vi) Endogenous chemical agents such as hypercholesterolaemia, endotoxins.
- vii) Exogenous chemical agents such as cigarette smoke.

2. ROLE OF PLATELETS. Following endothelial cell injury, platelets come to play a central role in normal haemostasis as well as in thrombosis. The sequence of events is as under:

i) Platelet adhesion. The platelets in circulation recognise the site of endothelial injury and adhere to exposed subendothelial collagen (*primary aggregation*), von Willebrand's factor is required for such adhesion between platelets and collagen. Normal non-activated platelets have open canalicular system with cytoplasmic organelles (granules, mitochondria, endoplasmic reticulum) dispersed throughout the cytoplasm (Fig. 17.1,A). During the early adhesion process, there is dilatation of canalicular system with formation of pseudopods and the cytoplasmic organelles shift to the centre of the cell (Fig. 17.1,B).

ii) Platelet release reaction. The activated platelets then undergo release reaction by which the platelet granules are released to the exterior (Fig. 17.1,C). Two main types of platelet granules are released:

a) *Alpha granules* containing fibrinogen, fibronectin, platelet-derived growth factor, platelet factor 4 (an anti-heparin) and cationic proteins.

b) *Dense bodies* containing ADP (adenosine diphosphate), ionic calcium, 5-HT (serotonin), histamine and epinephrine.

As a sequel to platelet activation and release reaction, the phospholipid complex-platelet factor 3 gets activated which plays important role in the intrinsic pathway of coagulation.

iii) Platelet aggregation. Following release of ADP, a potent platelet aggregating agent, aggregation of additional platelets takes place (*secondary aggregation*). This results in formation of temporary haemostatic plug. However, stable haemostatic plug is formed by the action of fibrin, thrombin and thromboxane A_2 .

3. ROLE OF COAGULATION SYSTEM. Coagulation mechanism is the conversion of the plasma fibrinogen into solid mass of fibrin. The coagulation system is involved in both haemostatic process and thrombus formation. Fig.17.2 shows the schematic representation of the cascade of intrinsic (blood) pathway, the extrinsic (tissue) pathway, and the common pathway leading to formation of fibrin polymers.

i) In the intrinsic pathway, contact with abnormal surface leads to activation of factor XII and the sequential

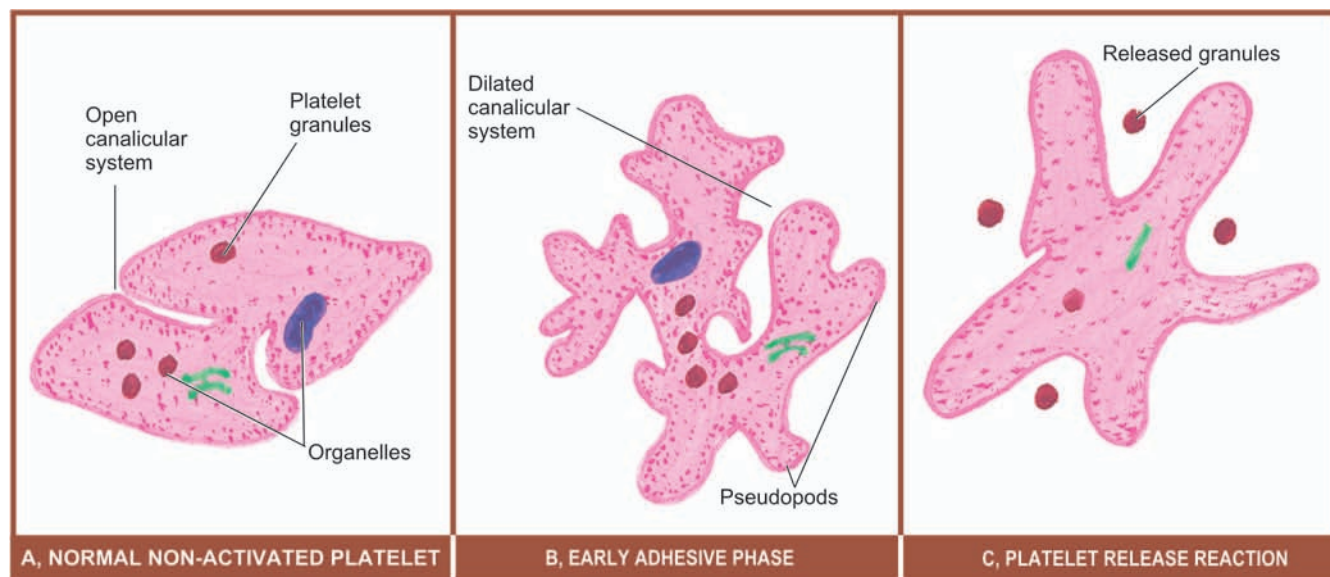


FIGURE 17.1

Activation of platelets during haemostatic plug formation and thrombogenesis. A, Normal non-activated platelet, having open canalicular system and the cytoplasmic organelles dispersed in the cell. B, Early adhesion phase, showing dilatation of the canalicular system with formation of pseudopods and the organelles present in the centre of the cell. C, Platelet release reaction, showing release of granules to the exterior.

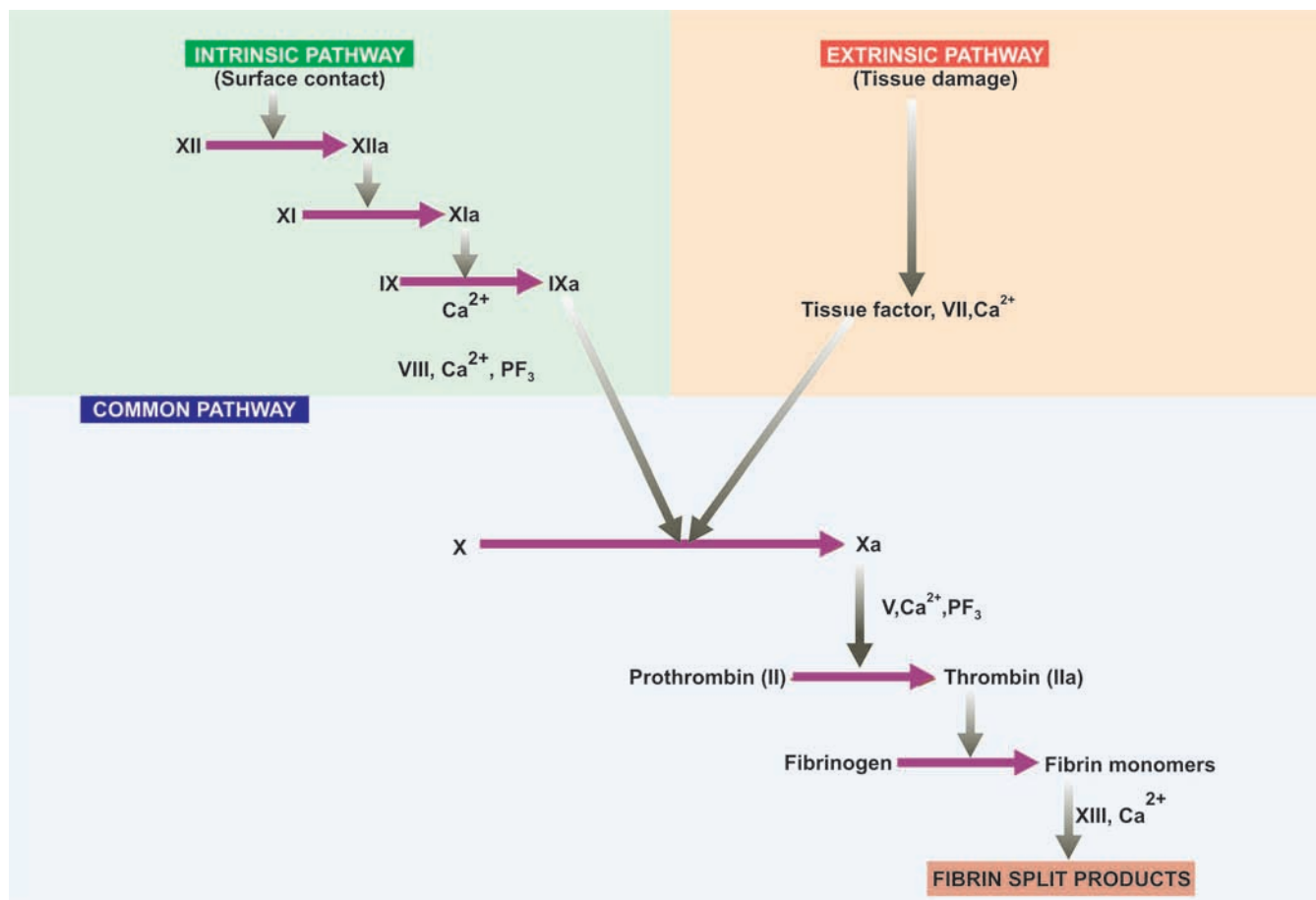


FIGURE 17.2

Schematic representation of pathways of coagulation mechanism.

interactions of factors XI, IX, VIII and finally factor X, alongwith calcium ions (factor IV) and platelet factor 3.

ii) In the extrinsic pathway, tissue damage results in the release of tissue factor or thromboplastin. Tissue factor on interaction with factor VII activates factor X.

iii) The common pathway begins where both intrinsic and extrinsic pathways converge to activate factor X which forms a complex with factor Va and platelet factor 3, in the presence of calcium ions. This complex activates prothrombin (factor II) to thrombin (factor IIa) which, in turn, converts fibrinogen to fibrin. Initial monomeric fibrin is polymerised to form insoluble fibrin by activation of factor XIII.

iv) Regulation of coagulation system. The blood is kept in fluid state normally and coagulation system kept in check by controlling mechanisms. These are as under:

a) *Protease inhibitors.* These act on coagulation factors so as to oppose the formation of thrombin e.g. anti-thrombin III, protein C, C₁ inactivator, α 1-antitrypsin,

α 2-macroglobulin.

b) *Fibrinolytic system.* Plasmin, a potent fibrinolytic enzyme, is formed by the action of plasminogen activator on plasminogen present in the normal plasma (Fig. 17.3). Two types of plasminogen activators (PA) are identified:

■ *Tissue-type PA* derived from endothelial cells and leucocytes.

■ *Urokinase-like PA* present in the plasma.

Plasmin so formed acts on fibrin to destroy the clot and produces fibrin split products (FSP).

4. HYPERCOAGULABILITY OF BLOOD. The occurrence of thrombosis in some conditions such as in nephrotic syndrome, advanced cancers, extensive trauma, burns and during puerperium is explained on the basis of hypercoagulability of blood. The effect of hypercoagulability on thrombosis is favoured by advancing age, smoking, use of oral contraceptives and obesity. Hypercoagulability may occur by the following changes

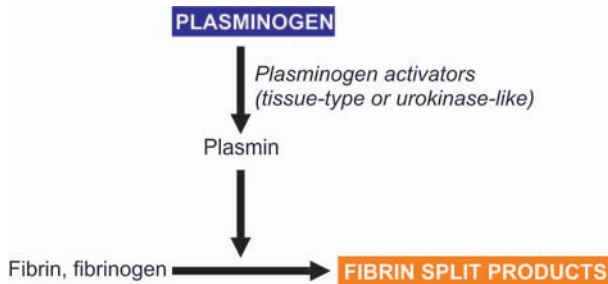


FIGURE 17.3

The fibrinolytic system.

in the composition of blood:

- i) Increase in coagulation factors e.g. fibrinogen, prothrombin, factor VIIa, VIIIa and Xa.
- ii) Increase in platelet count and their adhesiveness.
- iii) Decreased levels of coagulation inhibitors e.g. antithrombin III, fibrin split products.

5. ALTERATION OF BLOOD FLOW. Formation of arterial and cardiac thrombi is facilitated by turbulence in the blood flow, while stasis initiates the venous thrombi even without evidence of endothelial injury.

i) *Normally*, there is axial flow of blood in which the most rapidly-moving central stream consists of leucocytes and red cells. The platelets are present in the slow-moving laminar stream adjacent to the central stream while the peripheral stream consists of most slow-moving cell-free plasma close to endothelial layer (Fig. 17.4,A).

ii) *In turbulence and stasis*, the normal axial flow of blood is disturbed so that the platelets come into contact with the endothelium (Fig. 17.4,B).

Besides, the inhibitors of coagulation fail to reach the site of thrombus resulting in enlargement of thrombus size. Turbulence may actually injure the endothelium resulting in deposition of platelets and fibrin.

The sequence of events in thrombogenesis is illustrated in Fig. 17.5.

Morphology of Various Types

Thrombosis may occur in the heart, arteries, veins and the capillaries. Beside the differences in mechanisms of thrombus formation at these sites, the clinical effects of these are even more different. Arterial thrombi produce ischaemia and infarction, whereas cardiac and venous thrombi cause embolism.

The general morphologic features of thrombi are as under:

Grossly, thrombi may be of various shapes, sizes and

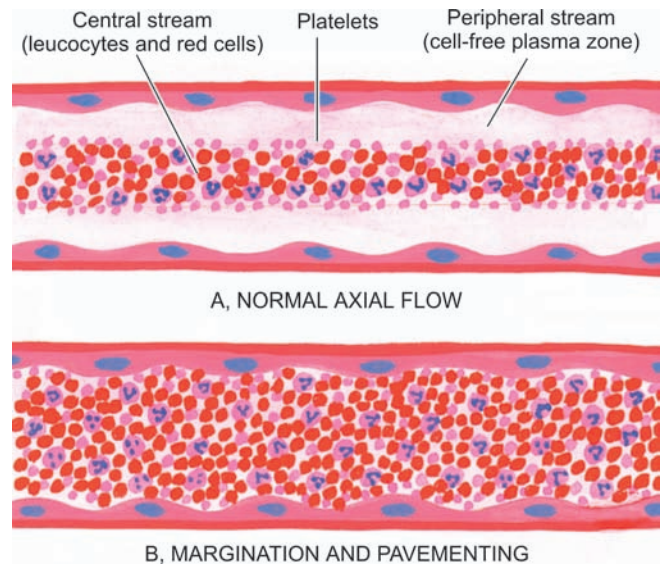


FIGURE 17.4

Alteration in flow of blood.

composition depending upon the site of origin. Arterial thrombi tend to be white and mural while the venous thrombi are red and occlusive. Mixed or laminated thrombi are also common and consist of alternate white and red layers called lines of Zahn. Red thrombi are soft, red and gelatinous whereas white thrombi are firm and pale.

Microscopically, the composition of thrombus is determined by the rate of flow of blood i.e. whether it is formed in the rapid arterial and cardiac circulation, or in the slow moving flow in veins. The lines of Zahn are formed by alternate layers of light-staining aggregated platelets admixed with fibrin meshwork and dark-staining layer of red cells. Red (venous) thrombi have more abundant red cells, leucocytes and platelets entrapped in fibrin meshwork (**COLOUR PLATE III: CL 11**). Thus, red thrombi closely resemble blood clots *in vitro* (Fig. 17.6).

Red thrombi (antemortem) have to be distinguished from postmortem clots (Table 17.1).

CARDIAC THROMBI. Thrombi may form in any of the chambers of heart and on the valve cusps. They are more common in the atrial appendages, especially of the right atrium, and on mitral and aortic valves called vegetations which may be seen in infective endocarditis and non-bacterial thrombotic endocarditis. Cardiac thrombi are mural (non-occlusive) as are the mural thrombi encountered in the aorta in atherosclerosis and in aneurysmal dilatations. Rarely, large round thrombus

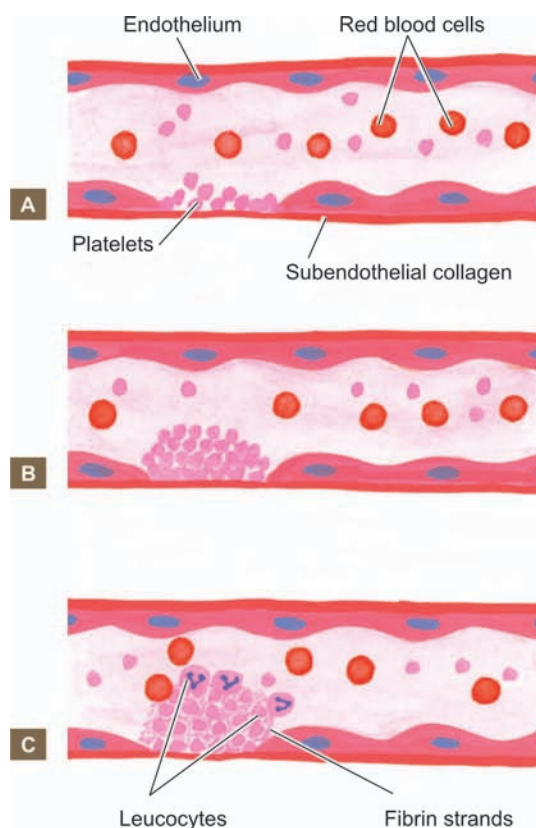


FIGURE 17.5

Sequence of events in thrombogenesis. A, Endothelial injury exposes subendothelium, initiating adherence of platelets and activation of coagulation system. B, Following platelet release reaction, ADP is released which causes further aggregation of platelets. C, Activated coagulation system forms fibrin strands in which are entangled some leucocytes and red cells.

may form and obstruct the mitral valve and is called *ball-valve thrombus*. *Agonal thrombi* are formed shortly before death and may occur in either or both the ventricles. They are composed mainly of fibrin.

ARTERIAL AND VENOUS THROMBI. The examples of major forms of vascular thrombi are as under:

Arterial thrombi:

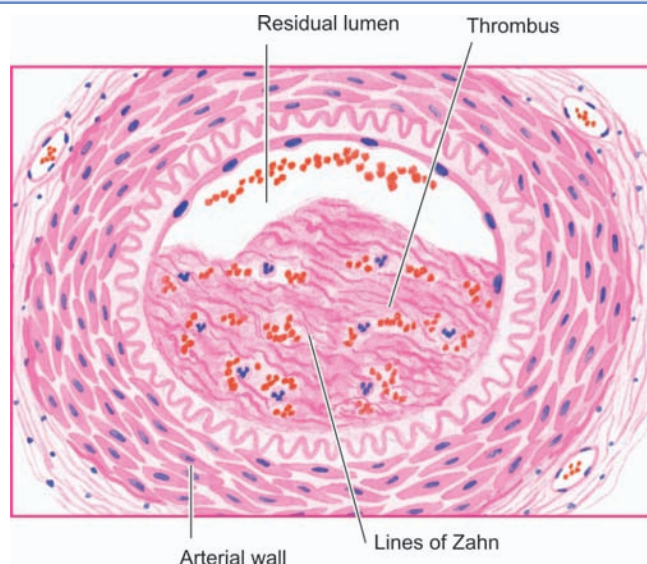


FIGURE 17.6

Thrombus in an artery. The thrombus is adherent to the arterial wall and is seen occluding most of the lumen. It shows lines of Zahn composed of granular-looking platelets and fibrin meshwork with entangled red cells and leucocytes.

- i) *Aorta*: aneurysms, arteritis.
- ii) *Coronary arteries*: atherosclerosis.
- iii) *Mesenteric artery*: atherosclerosis, arteritis.
- iv) *Arteries of limbs*: atherosclerosis, diabetes mellitus, Buerger's disease, Raynaud's disease.
- v) *Renal artery*: atherosclerosis, arteritis.
- vi) *Cerebral artery*: atherosclerosis, vasculitis.

Venous thrombi:

- i) *Veins of lower limbs*: deep veins of legs, varicose veins.
- ii) *Popliteal, femoral and iliac veins*: postoperative stage, postpartum.
- iii) *Pulmonary veins*: CHF, pulmonary hypertension.
- iv) *Hepatic and portal vein*: portal hypertension.
- v) *Superior vena cava*: infections in head and neck.
- vi) *Inferior vena cava*: extension of thrombus from hepatic vein.
- vii) *Mesenteric veins*: volvulus, intestinal obstruction.
- viii) *Renal vein*: renal amyloidosis.

TABLE 17.1: Distinguishing Features of Antemortem Thrombi and Postmortem Clots.

FEATURE	ANTEMORTEM THROMBI	POSTMORTEM CLOTS
1. <i>Gross</i>	Dry, granular, firm and friable	Gelatinous, soft and rubbery
2. <i>Relation to vessel wall</i>	Adherent to the vessel wall	Weakly attached to the vessel wall
3. <i>Shape</i>	May or may not fit their vascular contours	Take the shape of vessel or its bifurcation
4. <i>Microscopy</i>	The surface contains apparent lines of Zahn	The surface is 'chicken fat' yellow covering the underlying red 'currant jelly'

TABLE 17.2: Distinguishing Features of Arterial and Venous Thrombi.

FEATURE	ARTERIAL THROMBI	VENOUS THROMBI
1. <i>Blood flow</i>	Formed in rapidly-flowing blood of arteries and heart	Formed in slow-moving blood in veins
2. <i>Sites</i>	Common in aorta, coronary, cerebral, iliac, femoral, renal and mesenteric arteries	Common in superficial varicose veins, deep leg veins, popliteal, femoral and iliac veins
3. <i>Thrombogenesis</i>	Formed following endothelial cell injury e.g. in atherosclerosis	Formed following venous stasis e.g. in abdominal operations, child-birth
4. <i>Development</i>	Usually mural, not occluding the lumen completely, may propagate	Usually occlusive, take the cast of the vessel in which formed, may propagate in both directions
5. <i>Macroscopy</i>	Grey-white, friable with lines of Zahn on surface	Red-blue with fibrin strands and lines of Zahn
6. <i>Microscopy</i>	Distinct lines of Zahn composed of platelets, fibrin with entangled red and white blood cells	Lines of Zahn with more abundant red cells
7. <i>Effects</i>	Ischaemia leading to infarcts e.g. in the heart, brain etc	Thromboembolism, oedema, skin ulcers, poor wound healing

Distinguishing features between thrombi formed in rapidly-flowing arterial circulation and slow-moving venous blood are given in Table 17.2.

CAPILLARY THROMBI. Minute thrombi composed mainly of packed red cells are formed in the capillaries in acute inflammatory lesions, vasculitis and in disseminated intravascular coagulation (DIC).

Fate of Thrombus

The possible fate of thrombi is as under (Fig. 17.7):

1. RESOLUTION. Thrombus activates the fibrinolytic system with consequent release of plasmin which

may dissolve the thrombus completely resulting in resolution. Usually, lysis is complete in small venous thrombi while large thrombi may not be dissolved. Fibrinolytic activity can be accentuated by administration of thrombolytic substances (e.g. urokinase, streptokinase), especially in the early stage when fibrin is in monomeric form.

2. ORGANISATION. If the thrombus is not removed, it starts getting organised. Phagocytic cells (neutrophils and macrophages) appear and begin to phagocytose fibrin and cell debris. The proteolytic enzymes liberated by leucocytes and endothelial cells start digesting coagulum. Capillaries grow into the thrombus from the site of its

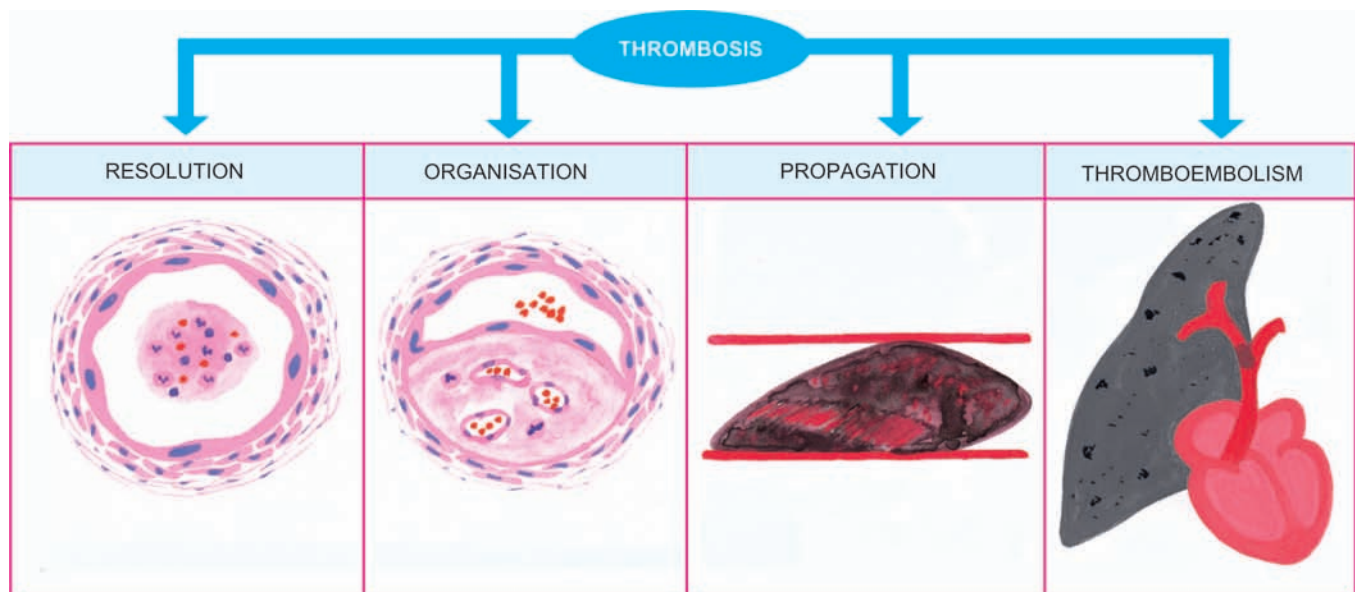


FIGURE 17.7

Fate of thrombus.

attachment and fibroblasts start invading the thrombus. Thus, fibrovascular granulation tissue is formed which subsequently becomes dense and less vascular and is covered over by endothelial cells. The thrombus in this way is excluded from the vascular lumen and becomes part of vessel wall. The new vascular channels in it may be able to re-establish the blood flow, called recanalisation. The fibrosed thrombus may undergo hyalinisation and calcification e.g. phleboliths in the pelvic veins.

3. PROPAGATION. The thrombus may enlarge in size due to more and more deposition from the constituents of flowing blood. In this way, it may ultimately cause obstruction of some important vessel.

4. THROMBOEMBOLISM. The thrombi in early stage and infected thrombi are quite friable and may get detached from the vessel wall. These are released in part or completely in bloodstream as emboli which produce ill-effects at the site of their lodgement (page 215).

Factors Predisposing to Thrombosis

A number of primary (genetic) and secondary (acquired) factors favour thrombosis.

Primary (Genetic) factors:

- i) Deficiency of antithrombin
- ii) Deficiency of protein C or S
- iii) Defects in fibrinolysis
- iv) Mutation in factor V

Secondary (acquired) factors:

- a) *Risk factors:*
 - i) Advanced age
 - ii) Prolonged bed-rest
 - iii) Immobilisation
 - iv) Use of oral contraceptives
 - v) Cigarette smoking
 - vi) Tissue damage e.g. trauma, fractures, burns
- b) *Clinical conditions predisposing to thrombosis:*
 - i) Heart diseases e.g. myocardial infarction, CHF, rheumatic mitral stenosis, cardiomyopathy.
 - ii) Atherosclerosis
 - iii) Aneurysms of the aorta and other vessels
 - iv) Varicosities of leg veins
 - v) Nephrotic syndrome
 - vi) Disseminated cancers
 - vii) Late pregnancy and puerperium.

Clinical Effects of Thrombosis

These depend upon the site of thrombi, rapidity of formation, and nature of thrombi.

1. Cardiac thrombi. Large thrombi in the heart may cause sudden death by mechanical obstruction of blood

flow or through thromboembolism to vital organs.

2. Arterial thrombi. These cause ischaemic necrosis of the deprived part (infarct) which may lead to gangrene. Sudden death may occur following thrombosis of coronary artery.

3. Venous thrombi (Phlebothrombosis). These may cause various effects such as:

- i) Thromboembolism
- ii) Oedema of area drained
- iii) Poor wound healing
- iv) Skin ulcer
- v) Painful thrombosed veins (thrombophlebitis)
- vi) Painful white leg (phlegmasia alba dolens) due to ileofemoral venous thrombosis in postpartum cases
- vii) Thrombophlebitis migrans in cancer.

4. Capillary thrombi. Microthrombi in microcirculation may give rise to disseminated intravascular coagulation (DIC).

EMBOLISM

Definition and Types

Embolism is the process of partial or complete obstruction of some part of the cardiovascular system by any mass carried in the circulation; the transported intravascular mass detached from its site of origin is called an *embolus*. Most usual forms of emboli (90%) are thromboemboli i.e. originating from thrombi or their parts detached from the vessel wall.

Emboli may be of various types:

A. Depending upon the matter in the emboli, they can be:

- i) *Solid* e.g. detached thrombi (thromboemboli), atheromatous material, tumour cell clumps, tissue fragments, parasites, bacterial clumps, foreign bodies.
- ii) *Liquid* e.g. fat globules, amniotic fluid, bone marrow.
- iii) *Gaseous* e.g. air, other gases.

B. Depending upon whether infected or not, they are called:

- i) *Bland*, when sterile.
- ii) *Septic*, when infected.

C. Depending upon the source of the emboli, they are classified as:

- i) *Cardiac emboli* from left side of heart e.g. emboli originating from atrium and atrial appendages, infarct in the left ventricle, vegetations of endocarditis.
- ii) *Arterial emboli* e.g. in systemic arteries in the brain, spleen, kidney, intestine.

- iii) *Venous emboli* e.g. in pulmonary arteries.
- iv) *Lymphatic emboli* can also occur.

D. Depending upon the flow of blood, two special types of emboli are mentioned:

i) *Paradoxical embolus*. An embolus which is carried from the venous side of circulation to the arterial side or vice versa is called paradoxical or crossed embolus e.g. through arteriovenous communication such as in patent foramen ovale, septal defect of the heart, and arteriovenous shunts in the lungs.

ii) *Retrograde embolus*. An embolus which travels against the flow of blood is called retrograde embolus e.g. metastatic deposits in the spine from carcinoma prostate. The spread occurs by retrograde embolism through intraspinal veins which carry tumour emboli from large thoracic and abdominal veins due to increased pressure in body cavities e.g. during coughing or straining.

Some of the important types of embolism are tabulated in Table 17.3 and described below:

Thromboembolism

A detached thrombus or part of thrombus constitutes the most common type of embolism. These may arise in the arterial or venous circulation (Fig. 17.8):

Arterial (systemic) thromboembolism. Arterial emboli

TABLE 17.3: Important Types of Embolism.

TYPE	COMMON ORIGIN
1. <i>Pulmonary embolism</i>	Veins of lower legs
2. <i>Systemic embolism</i>	Left ventricle (arterial)
3. <i>Fat embolism</i>	Trauma to bones/soft tissues
4. <i>Air embolism</i>	Venous: head & neck operations, obstetrical trauma Arterial: cardiothoracic surgery, angiography
5. <i>Decompression sickness</i>	Descent: divers Ascent: unpressurised flight
6. <i>Amniotic fluid embolism</i>	Components of amniotic fluid
7. <i>Atheroembolism</i>	Atheromatous plaques
8. <i>Tumour embolism</i>	Tumour fragments

may be derived from the following sources:

A. *Causes within the heart* (80-85%): These are mural thrombi in the left atrium or left ventricle, vegetations on the mitral or aortic valves, prosthetic heart valves and cardiomyopathy.

B. *Causes within the arteries*: These include emboli developing in relation to atherosclerotic plaques, aortic aneurysms, pulmonary veins and paradoxical arterial emboli from the systemic venous circulation.

The *effects* of arterial emboli depend upon their size, site of lodgement, and adequacy of collateral circulation.

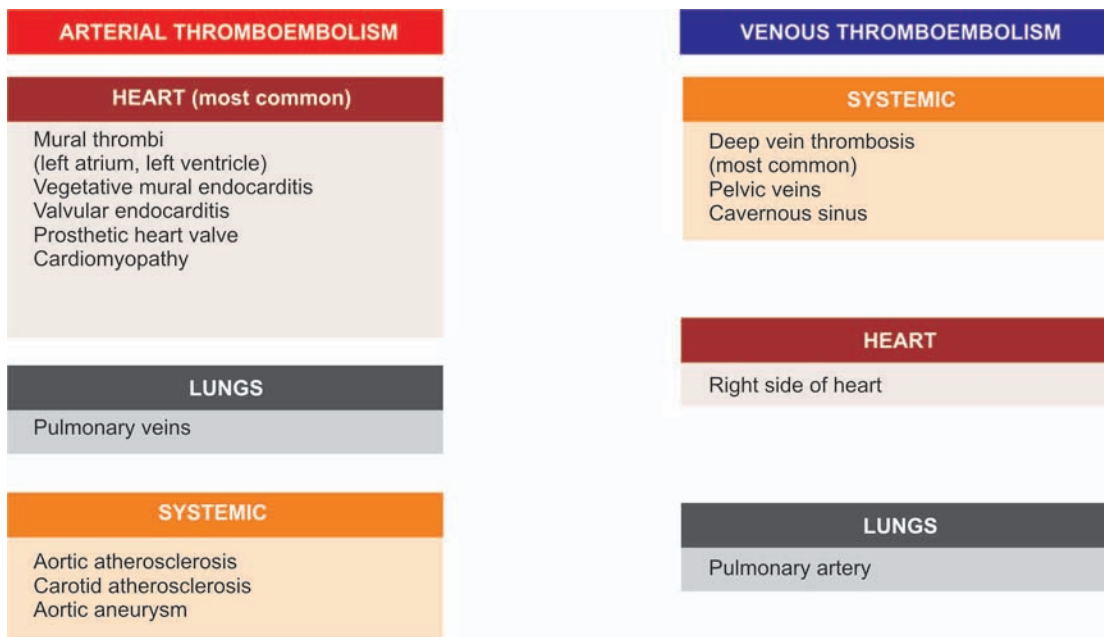


FIGURE 17.8

Sources of arterial and venous emboli.

If the vascular occlusion occurs, the following ill-effects may result:

- i) *Infarction* of the organ or its affected part e.g. ischaemic necrosis in the lower limbs (70-75%), spleen, kidneys, brain, intestine.
- ii) *Gangrene* following infarction in the lower limbs if the collateral circulation is inadequate.
- iii) *Arteritis and mycotic aneurysm* formation from bacterial endocarditis.
- iv) *Myocardial infarction* may occur following coronary embolism.
- v) *Sudden death* may result from coronary embolism or embolism in the middle cerebral artery.

Venous thromboembolism. Venous emboli may arise from the following sources:

- i) Thrombi in the veins of the lower legs are the most common cause of venous emboli.
- ii) Thrombi in the pelvic veins.
- iii) Thrombi in the veins of the upper limbs.
- iv) Thrombosis in cavernous sinus of the brain.
- v) Thrombi in the right side of heart.

The most significant *effect* of venous embolism is obstruction of pulmonary arterial circulation leading to pulmonary embolism described below.

Pulmonary Thromboembolism

DEFINITION. Pulmonary embolism is the most common and fatal form of venous thromboembolism in which there is occlusion of pulmonary arterial tree by thromboemboli. Pulmonary thrombosis as such is uncommon and may occur in pulmonary atherosclerosis and pulmonary hypertension. Differentiation of pulmonary thrombosis from pulmonary thromboembolism is tabulated in Table 17.4.

ETIOLOGY. Pulmonary emboli are more common in hospitalised or bed-ridden patients, though they can occur in ambulatory patients as well. The causes are as follows:

- i) Thrombi originating from large veins of lower legs (such as popliteal, femoral and iliac) are the cause in 95% of pulmonary emboli.
- ii) Less common sources include thrombi in varicosities of superficial veins of the legs, and pelvic veins such as peri-prostatic, periovarian, uterine and broad ligament veins.

PATHOGENESIS. Detachment of thrombi from any of the above-mentioned sites produces a thrombo-embolus that flows through venous drainage into the larger veins draining into right side of the heart.

■ If the thrombus is large, it is impacted at the bifurcation of the main pulmonary artery (*saddle embolus*), or may be found in the right ventricle or its outflow tract.

■ More commonly, there are *multiple emboli*, or a large embolus may be fragmented into many smaller emboli which are then impacted in a number of vessels, particularly affecting the lower lobes of lungs.

■ Rarely, *paradoxical embolism* may occur by passage of an embolus from right heart into the left heart through atrial or ventricular septal defect. In this way, pulmonary emboli may reach systemic circulation.

CONSEQUENCES OF PULMONARY EMBOLISM. Pulmonary embolism occurs more commonly as a complication in patients of acute or chronic debilitating diseases who are immobilised for a long duration. Women in their reproductive period are at higher risk such as in late pregnancy, following delivery and with use of contraceptive pills. The effects of pulmonary embolism depend mainly on the size of the occluded vessel, the number of emboli, and on the cardiovascular status of the patient. The following consequences can result (Fig. 17.9):

- i) **Sudden death.** Massive pulmonary embolism results in instantaneous death, without occurrence of chest pain or dyspnoea. However, if the death is somewhat delayed, the clinical features resemble myocardial infarction i.e. severe chest pain, dyspnoea and shock.

TABLE 17.4: Contrasting Features of Pulmonary Thrombosis and Pulmonary Thromboembolism.

FEATURE	PUL. THROMBOSIS	PUL. THROMBOEMBOLISM
1. <i>Pathogenesis</i>	Locally formed	Travelled from distance
2. <i>Location</i>	In small arteries and branches	In major arteries and branches
3. <i>Attachment to vessel wall</i>	Firmly adherent	Loosely attached or lying free
4. <i>Gross appearance</i>	Head pale, tail red	No distinction in head and tail; smooth surface dry dull surface
5. <i>Microscopy</i>	Platelets and fibrin in layers, Lines of Zahn seen	Mixed with blood clot, lines of Zahn rare

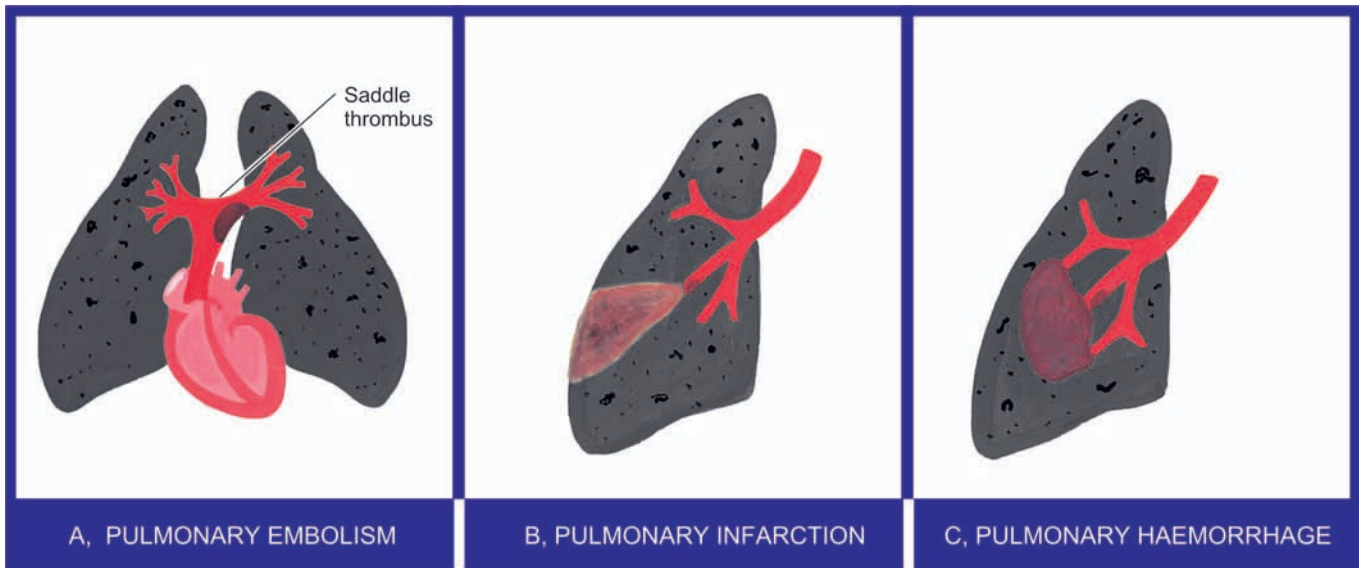


FIGURE 17.9

Three main consequences of pulmonary embolism. A, Saddle embolus, causing sudden death. B, Pulmonary infarction, commonly peripheral. C, Pulmonary haemorrhage, usually central.

ii) Acute cor pulmonale. Numerous small emboli may obstruct most of the pulmonary circulation resulting in acute right heart failure. Another mechanism is by release of vasoconstrictor substances from platelets or by reflex vasoconstriction of pulmonary vessels.

iii) Pulmonary infarction. Obstruction of relatively small-sized pulmonary arterial branches may result in pulmonary infarction (page 227). The clinical features include chest pain due to fibrinous pleuritis, haemoptysis and dyspnoea due to reduced functioning pulmonary parenchyma.

iv) Pulmonary haemorrhage. Obstruction of terminal branches (endarteries) leads to central pulmonary haemorrhage. The clinical features are haemoptysis, dyspnoea, and less commonly, chest pain due to central location of pulmonary haemorrhage. Sometimes, there may be concomitant pulmonary infarction.

v) Resolution. Vast majority of small pulmonary emboli (60-80%) are resolved by fibrinolytic activity. These patients are clinically silent owing to bronchial circulation so that lung parenchyma is adequately perfused.

vi) Pulmonary hypertension, chronic cor pulmonale and pulmonary arteriosclerosis. These are the sequelae of multiple small thromboemboli undergoing healing rather than resolution.

Systemic Embolism

This is the type of arterial embolism that originates commonly from thrombi in the diseased heart, especially in

the left ventricle. These diseases of heart include myocardial infarction, cardiomyopathy, RHD, congenital heart disease, infective endocarditis, and prosthetic cardiac valves. These arterial emboli invariably cause infarction at the sites of lodgement which include, in descending order of frequency, lower extremity, brain, and internal visceral organs (spleen, kidneys, intestines). *Thus, the effects and sites of arterial emboli are in striking contrast to venous emboli which are often lodged in the lungs.*

Fat Embolism

Obstruction of arterioles and capillaries by fat globules constitutes fat embolism. If the obstruction in the circulation is by fragments of adipose tissue, it is called fat-tissue embolism.

ETIOLOGY. Following are the important causes of fat embolism:

i) Traumatic causes:

■ *Trauma to bones* is the most common cause of fat embolism e.g. in fractures of long bones leading to passage of fatty marrow in circulation, concussions of bones, after orthopaedic surgical procedures etc.

■ *Trauma to soft tissue* e.g. laceration of adipose tissue and in puerperium due to injury to pelvic fatty tissue.

ii) Non-traumatic causes:

- Extensive burns
- Diabetes mellitus

- Fatty liver
- Pancreatitis
- Sick cell anaemia
- Decompression sickness
- Inflammation of bones and soft tissues
- Extrinsic fat or oils introduced into the body.

PATHOGENESIS. The following mechanisms are proposed to explain the pathogenesis of fat embolism. These may be acting singly or in combination.

i) Mechanical theory. Mobilisation of fluid fat may occur following trauma to the bone or soft tissues. The fat globules released from the injured area may enter venous circulation and finally most of the fat is arrested in the small vessels in the lungs. Some of the fat globules may further pass through into the systemic circulation to lodge in other organs.

ii) Emulsion instability theory. This theory explains the pathogenesis of fat embolism in non-traumatic cases. According to this theory, fat emboli are formed by aggregation of plasma lipids (chylomicrons and fatty acids) due to disturbance in natural emulsification of fat.

iii) Intravascular coagulation theory. In stress, release of some factor activates disseminated intravascular coagulation (DIC) and aggregation of fat emboli.

iv) Toxic injury theory. According to this theory, the small blood vessels of lungs are chemically injured by high plasma levels of free fatty acid, resulting in increased vascular permeability and consequent pulmonary oedema.

CONSEQUENCES OF FAT EMBOLISM. The effects of fat embolism depend upon the size and quantity of fat globules, and whether or not the emboli pass through the lungs into the systemic circulation.

i) Pulmonary fat embolism. In patients dying after fractures of bones, presence of numerous fat emboli in the capillaries of the lung is a frequent autopsy finding because the small fat globules are not likely to appreciably obstruct the vast pulmonary vascular bed. However, widespread obstruction of pulmonary circulation due to extensive pulmonary embolism can occur and result in sudden death.

Microscopically, the lungs show hyperaemia, oedema, petechial haemorrhages and changes of adult respiratory distress syndrome (ARDS). Pulmonary infarction is usually not a feature of fat embolism because of the small size of globules. In routine stains, the fat globules in the pulmonary arteries, capillaries

and alveolar spaces appear as vacuoles. Frozen section is essential for confirmation of globules by fat stains such as Sudan dyes (Sudan black, Sudan III and IV), oil red O and osmic acid.

ii) Systemic fat embolism. Some of the fat globules may pass through the pulmonary circulation such as via patent foramen ovale, arteriovenous shunts in the lungs and vertebral venous plexuses, and get lodged in the capillaries of organs like the brain, kidney, skin etc.

■ **Brain.** The pathologic findings in the brain are petechial haemorrhages on the leptomeninges and minute haemorrhages in the parenchyma.

Microscopically, microinfarct of brain, oedema and haemorrhages are seen. The CNS manifestations include delirium, convulsions, stupor, coma and sudden death.

■ **Kidney.** Renal fat embolism present in the glomerular capillaries, may cause decreased glomerular filtration. Other effects include tubular damage and renal insufficiency.

■ **Other organs.** Besides the brain and kidneys, other findings in systemic fat embolism are petechiae in the skin, conjunctivae, serosal surfaces, fat globules in the urine and sputum.

Gas Embolism

Air, nitrogen and other gases can produce bubbles within the circulation and obstruct the blood vessels causing damage to tissue. Two main forms of gas embolism—air embolism and decompression sickness are described below.

Air Embolism

Air embolism occurs when air is introduced into venous or arterial circulation.

VENOUS AIR EMBOLISM. Air may be sucked into systemic veins under the following circumstances:

i) Operations on head and neck, and trauma. The accidental opening of a major vein of the neck like jugular, or neck wounds involving the major neck veins, may allow air to be drawn into venous circulation.

ii) Obstetrical operations and trauma. During childbirth by normal vaginal delivery, caesarean section, abortions and other procedures, fatal air embolism may result from the entrance of air into the opened-up uterine venous sinuses and endometrial veins.

iii) Intravenous infusion of blood and fluid. Air embolism may occur during intravenous blood or fluid infusions if only positive pressure is employed.

iv) Angiography. During angiographic procedures, air may be entrapped into a large vein causing air embolism.

The **effects** of venous air embolism depend upon the following factors:

- i) *Amount of air* introduced into the circulation. The volume of air necessary to cause death is variable but usually 100-150 ml of air entry is considered fatal.
- ii) *Rapidity* of entry of a smaller volume of air is important determinant of a fatal outcome.
- iii) *Position of the patient* during or soon after entry of air is another factor. The air bubbles may ascend into the superior vena cava if the position of head is higher than the trunk (e.g. in upright position) and reach the brain.
- iv) *General condition* of the patient e.g. in severely ill patients, as little as 40 ml of air may have serious results.

The mechanism of death is by entrapment of air emboli in the pulmonary arterial trunk in the right heart. If bubbles of air in the form of froth pass further out into pulmonary arterioles, they cause widespread vascular occlusions. If death from pulmonary air embolism is suspected, the heart and pulmonary artery should be opened *in situ* under water so that escaping froth or foam formed by mixture of air and blood can be detected.

ARTERIAL AIR EMBOLISM. Entry of air into pulmonary vein or its tributaries may occur in the following conditions:

- i) **Cardiothoracic surgery and trauma.** Arterial air embolism may occur following thoracic operations, thoracocentesis, rupture of the lung, penetrating wounds of the lung, artificial pneumothorax etc.
- ii) **Paradoxical air embolism.** This may occur due to passage of venous air emboli to the arterial side of circulation through a patent foramen ovale or via pulmonary arteriovenous shunts.
- iii) **Arteriography.** During arteriographic procedures, air embolism may occur.

The **effects** of arterial air embolism are in the form of certain characteristic features:

- i) Marble skin due to blockage of cutaneous vessels.
- ii) Air bubbles in the retinal vessels seen ophthalmoscopically.
- iii) Pallor of the tongue due to occlusion of a branch of lingual artery.
- iv) Coronary or cerebral arterial air embolism may cause sudden death by much smaller amounts of air than in the venous air embolism.

Decompression Sickness

This is a specialised form of gas embolism known by

various names such as caisson's disease, divers' palsy or aeroembolism.

PATHOGENESIS. Decompression sickness is produced when the individual decompresses suddenly, either from high atmospheric pressure to normal level, or from normal pressure to low atmospheric pressure.

■ In divers, workers in caissons (diving-bells), offshore drilling and tunnels, who *descend* to high atmospheric pressure, increased amount of atmospheric gases (mainly nitrogen; others are O₂, CO₂) are dissolved in blood and tissue fluids. When such an individual ascends too rapidly i.e. comes to normal level suddenly from high atmospheric pressure, the gases come out of the solution as minute bubbles, particularly in fatty tissues which have affinity for nitrogen. These bubbles may coalesce together to form large emboli.

■ In aeroembolism, seen in those who *ascend* to high altitudes or air flight in unpressurised cabins, the individuals are exposed to sudden decompression from low atmospheric pressure to normal levels. This results in similar effects as in divers and workers in caissons.

EFFECTS. The effects of decompression sickness depend upon the following:

- Depth or altitude reached
- Duration of exposure to altered pressure
- Rate of ascent or descent
- General condition of the individual

The pathologic changes are more pronounced in sudden decompression from high pressure to normal levels than in those who decompress from low pressure to normal levels. The changes are more serious in obese persons as nitrogen gas is more soluble in fat than in body fluids.

Clinical effects of decompression sickness are of 2 types—acute and chronic.

■ **Acute form** occurs due to acute obstruction of small blood vessels in the vicinity of joints and skeletal muscles. The condition is clinically characterised by the following:

- i) 'The bends', as the patient doubles up in bed due to acute pain in joints, ligaments and tendons.
- ii) 'The chokes' occur due to accumulation of bubbles in the lungs, resulting in acute respiratory distress.
- iii) Cerebral effects may manifest in the form of vertigo, coma, and sometimes death.

■ **Chronic form** is due to foci of ischaemic necrosis throughout body, especially the skeletal system. Ischaemic necrosis may be due to embolism *per se*, but other factors such as platelet activation, intravascular coagulation and hypoxia might contribute. The features

of chronic form are as under:

- i) *Avascular necrosis of bones* e.g. head of femur, tibia, humerus.
- ii) *Neurological symptoms* may occur due to ischaemic necrosis in the central nervous system. These include paraesthesias and paraplegia.
- iii) *Lung involvement* in the form of haemorrhage, oedema, emphysema and atelectasis may be seen. These result in dyspnoea, nonproductive cough and chest pain.
- iv) *Skin manifestations* include itching, patchy erythema, cyanosis and oedema.
- v) *Other organs* like parenchymal cells of the liver and pancreas may show lipid vacuoles.

Amniotic Fluid Embolism

This is the most serious, unpredictable and unpreventable cause of maternal mortality. During labour and in the immediate postpartum period, the contents of amniotic fluid may enter the uterine veins and reach right side of the heart resulting in fatal complications. The amniotic fluid components which may be found in uterine veins, pulmonary artery and vessels of other organs are: epithelial squames, vernix caseosa, lanugo hair, bile from meconium, and mucus. The mechanism by which these amniotic fluid contents enter the maternal circulation is not clear. Possibly, they gain entry either through tears in the myometrium and endocervix, or the amniotic fluid is forced into uterine sinusoids by vigorous uterine contractions.

PATHOLOGIC CHANGES. Notable changes are seen in the lungs such as haemorrhages, congestion, oedema and changes of ARDS, and dilatation of right side of the heart.

These changes are associated with identifiable amniotic fluid contents within the pulmonary microcirculation.

The *clinical syndrome* is characterised by the following features:

- Sudden respiratory distress and dyspnoea
- Deep cyanosis
- Cardiovascular shock
- Convulsions
- Coma
- Unexpected death

The *cause of death* may not be obvious but can occur as a result of the following mechanisms:

- i) Mechanical blockage of the pulmonary circulation in

extensive embolism.

- ii) Anaphylactoid reaction to amniotic fluid components.
- iii) Disseminated intravascular coagulation (DIC) due to liberation of thromboplastin by amniotic fluid.
- iv) Haemorrhagic manifestations due to thrombocytopenia and afibrinogenaemia.

Atheroembolism

Atheromatous plaques, especially from aorta, may get eroded to form atherosclerotic emboli which are then lodged in medium-sized and small arteries. These emboli consist of cholesterol crystals, hyaline debris and calcified material, and may evoke foreign body reaction at the site of lodgement.

PATHOLOGIC CHANGES and their effects in atheroembolism are as under:

- i) Ischaemia, atrophy and necrosis of tissue distal to the occluded vessel.
- ii) Infarcts in the organs affected such as the kidneys, spleen, brain and heart.
- iii) Gangrene in the lower limbs.
- iv) Hypertension, if widespread renal vascular lesions are present.

Tumour Embolism

Malignant tumour cells invade the local blood vessels and may form tumour emboli to be lodged elsewhere, producing metastatic tumour deposits. Notable examples are clear cell carcinoma of kidney, carcinoma of the lung, malignant melanoma etc (Chapter 20).

Miscellaneous Emboli

Various other endogenous and exogenous substances may act as emboli. These are:

- i) Fragments of tissue
- ii) Placental fragments
- iii) Red cell aggregates (sludging)
- iv) Bacteria
- v) Parasites
- vi) Barium emboli following enema
- vii) Foreign bodies e.g. needles, talc, sutures, bullets, catheters etc.



ISCHAEMIA

DEFINITION. Ischaemia is defined as deficient blood supply to part of a tissue. The cessation of blood supply may be complete (complete ischaemia) or partial (partial ischaemia). The harmful effects of ischaemia may result from 3 ways:

1. Hypoxia due to deprivation of oxygen to tissues.
2. Inadequate supply of nutrients to the tissue such as glucose and amino acids.
3. Inadequate clearance of metabolites resulting in accumulation of metabolic waste-products in the affected tissue.

ETIOLOGY. A number of causes may produce ischaemia. These are as under:

1. Causes in the heart. Inadequate cardiac output resulting from heart block, ventricular arrest and fibrillation may cause hypoxic injury to brain.

- If the arrest continues for 15 seconds, consciousness is lost.
- If the condition lasts for more than 4 minutes, irreversible ischaemic damage to brain occurs.
- If it is prolonged for more than 8 minutes, death is inevitable.

2. Causes in the arteries. The commonest and most important causes of ischaemia are due to obstruction in arterial blood supply. These are:

i) *Luminal occlusion* such as due to:

- Thrombosis
- Embolism

ii) *Causes in the arterial wall* such as:

- Vasospasm (e.g. in Raynaud's disease)
- Hypothermia, ergotism
- Arteriosclerosis
- Polyarteritis nodosa
- Thromboangiitis obliterans (Buerger's disease)
- Severed vessel wall

iii) *Outside pressure* on an artery such as:

- Ligature
- Tourniquet
- Tight plaster, bandages
- Torsion.

3. Causes in the veins. Blockage of venous drainage may lead to engorgement and obstruction to arterial blood supply resulting in ischaemia. The examples include the following:

i) *Luminal occlusion* such as in:

- Thrombosis of mesenteric veins
- Cavernous sinus thrombosis

ii) *Causes in the vessel wall* such as in:

- Varicose veins of the legs

iii) *Outside pressure* on a vein as in:

- Strangulated hernia
- Intussusception
- Volvulus

4. Causes in the microcirculation. Ischaemia may result from occlusion of arterioles, capillaries and venules. The causes are as under:

i) *Luminal occlusion* such as:

- By red cells e.g. in sickle cell anaemia, red cells parasitised by malaria, acquired haemolytic anaemia, sludging of the blood.
- By white cells e.g. in chronic myeloid leukaemia
- By fibrin e.g. defibrination syndrome
- By precipitated cryoglobulins
- By fat embolism
- In decompression sickness.

ii) *Causes in the microvasculature wall* such as:

- Vasculitis e.g. in polyarteritis nodosa, Henoch-Schönlein purpura, Arthus reaction, septicaemia.
- Frost-bite injuring the wall of small blood vessels.

iii) *Outside pressure on microvasculature* as in:

- Bedsores.

FACTORS DETERMINING THE SEVERITY OF ISCHAEMIC INJURY. The extent of damage produced by ischaemia due to occlusion of arterial or venous blood vessels depends upon a number of factors. These are as under:

1. Anatomic pattern. The extent of injury by ischaemia depends upon the anatomic pattern of arterial blood supply of the organ or tissue affected. There are 4 different patterns of arterial blood supply:

i) *Single arterial supply without anastomosis.* Some organs receive blood supply from arteries which do not have significant anastomosis and are thus functional end-arteries. Occlusion of such vessels invariably results in ischaemic necrosis. The examples are:

- Central artery of the retina
- Interlobular arteries of the kidneys.

ii) *Single arterial supply with rich anastomosis.* Arterial supply to some organs has rich interarterial anastomoses so that blockage of one vessel can re-establish blood supply bypassing the blocked arterial branch, and hence the infarction is less common in such circumstances. For example:

- Superior mesenteric artery supplying blood to the small intestine.
- Inferior mesenteric artery supplying blood to distal colon.
- Arterial supply to the stomach by 3 separate vessels derived from coeliac axis.
- Interarterial anastomoses in the 3 main trunks of the coronary arterial system.

iii) *Parallel arterial supply.* Blood supply to some organs and tissues is such that the vitality of the tissue is maintained by alternative blood supply in case of occlusion of one. The examples are:

- Blood supply to brain in the region of circle of Willis.
- Arterial supply to forearm by radial and ulnar arteries.

iv) *Double blood supply.* The effect of occlusion of one set of vessels is modified if an organ has dual blood supply. For example:

- Lungs are perfused by bronchial circulation as well as by pulmonary arterial branches.
- Liver is supplied by both portal circulation and hepatic arterial flow.

However, collateral circulation is of little value if the vessels are severely affected with spasm, atheroma or any other condition.

2. General and cardiovascular status. The general status of an individual as regards cardiovascular function is an important determinant to assess the effect of ischaemia. Some of the factors which render the tissues more vulnerable to the effects of ischaemia are:

- i) Anaemias (sickle cell anaemia, in particular)
- ii) Lowered oxygenation of blood (hypoxaemia)
- iii) Senility with marked coronary atherosclerosis
- iv) Cardiac failure
- v) Blood loss
- vi) Shock.

3. Type of tissue affected. The vulnerability of tissue of the body to the effect of ischaemia is variable. The

mesenchymal tissues are quite resistant to the effect of ischaemia as compared to parenchymal cells of the organs. The following tissues are more vulnerable to ischaemia:

- i) Brain (cerebral cortical neurons, in particular).
- ii) Heart (myocardial cells).
- iii) Kidney (especially epithelial cells of proximal convoluted tubules).

4. Rapidity of development. Sudden vascular obstruction results in more severe effects of ischaemia than if it is gradual since there is less time for collaterals to develop.

5. Degree of vascular occlusion. Complete vascular obstruction results in more severe ischaemic injury than the partial occlusion.

EFFECTS. The effects of ischaemia are variable and range from 'no change' to 'sudden death'.

1. No effects on the tissues, if the collateral channels develop adequately so that the effect of ischaemia fails to occur.

2. Functional disturbances. These result when collateral channels are able to supply blood during normal activity but the supply is not adequate to withstand the effect of exertion. The examples are angina pectoris and intermittent claudication.

3. Cellular changes. Partial ischaemia may produce cellular changes such as cloudy swelling, fatty change, atrophy and replacement fibrosis. Infarction results when the deprivation of blood supply is complete so as to cause necrosis of tissue affected.

4. Sudden death. The cause of sudden death from ischaemia is usually myocardial and cerebral infarction.

INFARCTION

DEFINITION. Infarction is the process of tissue necrosis resulting from some form of circulatory insufficiency; the localised area of necrosis so developed is called an *infarct*.

ETIOLOGY. All the causes of ischaemia discussed above can cause infarction. There are a few other note-worthy features in infarcts:

- Most commonly, infarcts are caused by interrupted arterial blood supply, called *ischaemic necrosis*.
- Less commonly, venous obstruction can produce infarcts termed *stagnant hypoxia*.
- Generally, *sudden, complete, and continuous occlusion* by thrombosis or embolism produces infarcts.
- Infarcts may be produced by *nonocclusive circulatory insufficiency* as well e.g. incomplete atherosclerotic

narrowing of coronary arteries may produce myocardial infarction due to acute coronary insufficiency.

TYPES OF INFARCTS. Infarcts are classified depending upon different features:

1. **According to their colour,** infarcts may be:

- *Pale or anaemic*, due to arterial occlusion and are seen in compact organs e.g. in the kidneys, heart, spleen.
- *Red or haemorrhagic*, seen in soft loose tissues and are caused either by pulmonary arterial obstruction (e.g. in the lungs) or by arterial or venous occlusion (e.g. in the intestines).

2. **According to their age,** infarcts are classified as:

- *Recent or fresh*
- *Old or healed*

3. **According to presence or absence of infection,** they may be:

- *Bland*, when free of bacterial contamination
- *Septic*, when infected.

PATHOGENESIS. The process of infarction takes place as follows:

- i) *Localised hyperaemia* due to local anoxaemia occurs immediately after obstruction of the blood supply.
- ii) Within a few hours, the affected part becomes swollen due to *oedema and haemorrhage*. The amount of haemorrhage is variable, being more marked in the lungs and spleen, and less extensive in the kidneys and heart.
- iii) *Cellular changes* such as cloudy swelling and degeneration appear early, while death of the cells or necrosis occurs in 12-48 hours.
- iv) There is progressive *autolysis* of the necrotic tissue and haemolysis of the red cells.
- v) *An acute inflammatory reaction and hyperaemia* appear at the same time in the surrounding tissues in response to products of autolysis.
- vi) *Blood pigments*, haematoidin and haemosiderin, liberated by haemolysis are deposited in the infarct. At this stage, most infarcts become pale due to loss of red cells.
- vii) Following this, there is progressive *ingrowth of granulation tissue* from the margin of the infarct so that eventually the infarct is replaced by a fibrous scar. Dystrophic calcification may occur sometimes. However, in the case of infarct brain, there is liquefactive necrosis which heals by gliosis.

PATHOLOGIC CHANGES. Some general morphological features of infarcts are described below,

followed by pathologic changes in infarcts of different organs.

Grossly, infarcts of solid organs are usually wedge-shaped, the apex pointing towards the occluded artery and the wide base on the surface of the organ. Infarcts due to arterial occlusion are generally pale while those due to venous obstruction are haemorrhagic. Most infarcts become pale later as the red cells are lysed but pulmonary infarcts never become pale due to extensive amount of blood. Cerebral infarcts are poorly defined with central softening (encephalomalacia). Recent infarcts are generally slightly elevated over the surface while the old infarcts are shrunken and depressed under the surface of the organ.

Microscopically, the pathognomonic cytologic change in all infarcts is coagulative necrosis of the affected area of tissue or organ. In cerebral infarcts, however, there is characteristic liquefactive necrosis. Some amount of haemorrhage is generally present in any infarct. At the periphery of an infarct, inflammatory reaction is noted. Initially, neutrophils predominate but subsequently macrophages and fibroblasts appear. Eventually, the necrotic area is replaced by fibrous scar tissue, which at times may show dystrophic calcification. In cerebral infarcts, the liquefactive necrosis is followed by gliosis i.e. replacement by microglial cells distended by fatty material (gitter cells).

INFARCTS OF DIFFERENT ORGANS

Table 18.1 summarises the gross appearance and the usual outcome of the common types of infarction. Fig. 18.1 shows the organs most commonly affected by infarction.

A few representative examples of infarction of some organs (brain, intestines, lungs, kidney, liver and spleen) are discussed below, while myocardial infarction discussed later in Chapter 24.

CEREBRAL INFARCTION

Cerebral infarction is a localised area of tissue necrosis caused by local vascular occlusion—arterial or venous. Occasionally, it may be the result of non-occlusive causes such as compression on the cerebral arteries from outside and from hypoxic encephalopathy. Clinically, the signs and symptoms associated with cerebral infarction depend upon the region infarcted. In general, the focal neurologic deficit termed stroke, is present.

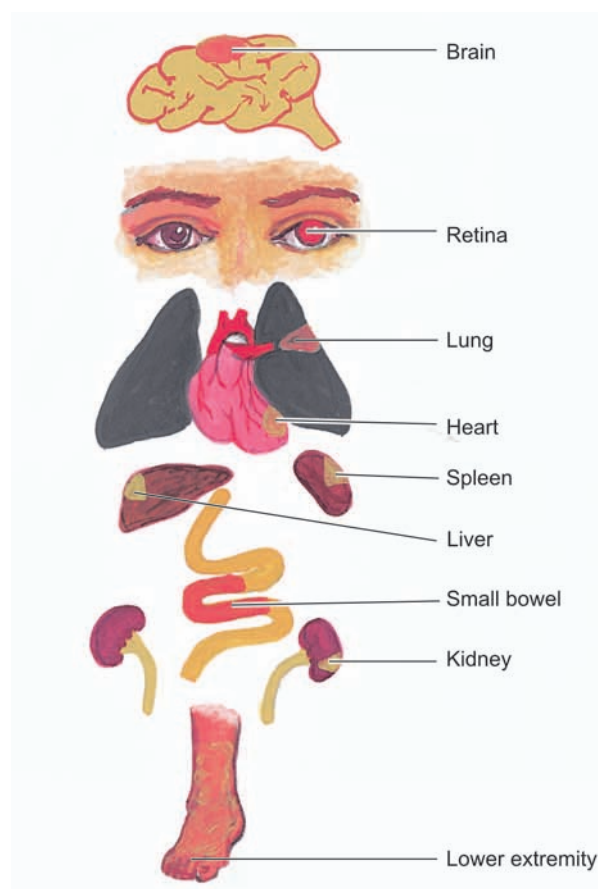


FIGURE 18.1

Common locations of systemic infarcts following arterial embolism.

However, significant atherosclerotic cerebrovascular disease may produce transient ischaemic attacks (TIA).

1. Arterial occlusion. Occlusion of the cerebral arteries by either thrombi or emboli is the most common cause of cerebral infarction. Thrombotic occlusion of the cerebral arteries is most frequently the result of atherosclerosis, and rarely, from arteritis of the cranial arteries.

Embolic arterial occlusion is commonly derived from the heart, most often from mural thrombosis complicating myocardial infarction, from atrial fibrillation and endocarditis. The size and shape of an infarct are determined by the extent of anastomotic connections with adjacent arterial branches. For instance,

■ The circle of Willis provides a complete collateral flow for internal carotid and vertebral arteries.

■ The middle and anterior cerebral arteries have partial anastomosis of their distal branches. Their complete occlusion may cause infarcts.

■ The small terminal cerebral arteries, on the contrary, are end-arteries and have no anastomosis. Hence, occlusion of these branches will invariably lead to an infarct.

2. Venous occlusion. Venous infarction in the brain is an infrequent phenomenon due to good communications of the cerebral venous drainage. However in cancer, due to increased predisposition to thrombosis, superior sagittal thrombosis may occur leading to bilateral, parasagittal, multiple haemorrhagic infarcts.

3. Non-occlusive causes. Compression of the cerebral arteries from outside such as occurs during herniation may cause cerebral infarction. Mechanism of watershed (border zone) cerebral infarction in hypoxic encephalopathy has already been explained above.

In any case, the extent of damage produced by any of the above causes depends upon:

- rate of reduction of blood flow;
- type of blood vessel involved; and
- extent of collateral circulation.

PATHOLOGIC CHANGES. Cerebral infarcts may be anaemic or haemorrhagic.

Grossly, an *anaemic infarct* becomes evident 6-12 hours after its occurrence. The affected area is soft and swollen and there is blurring of junction between grey

TABLE 18.1: Infarcts of Most Commonly Affected Organs.

LOCATION	GROSS APPEARANCE	OUTCOME
1. <i>Myocardial infarction</i>	Pale	Frequently lethal
2. <i>Pulmonary infarction</i>	Haemorrhagic	Less commonly fatal
3. <i>Cerebral infarction</i>	Haemorrhagic or pale	Fatal if massive
4. <i>Intestinal infarction</i>	Haemorrhagic	Frequently lethal
5. <i>Renal infarction</i>	Pale	Not lethal unless massive and bilateral
6. <i>Infarct spleen</i>	Pale	Not lethal
7. <i>Infarct liver</i>	Pale	Not lethal
8. <i>Infarcts lower extremity</i>	Pale	Not lethal

and white matter. Within 2-3 days, the infarct undergoes softening and disintegration. Eventually, there is central liquefaction with peripheral firm glial reaction and thickened leptomeninges, forming a cystic infarct (Fig. 18.2). A *haemorrhagic infarct* is red and superficially resembles a haematoma. It is usually the result of fragmentation of occlusive arterial emboli or venous thrombosis.

Microscopically, the sequential changes are as under:

1. Initially, there is eosinophilic neuronal necrosis and lipid vacuolisation produced by breakdown of myelin. Simultaneously, the infarcted area is infiltrated by neutrophils.
2. After the first 2-3 days, there is progressive invasion by macrophages and there is astrocytic and vascular proliferation.
3. In the following weeks to months, the macrophages clear away the necrotic debris by phagocytosis followed by reactive astrocytosis, often with little fine fibrosis (Fig. 18.3). A haemorrhagic infarct has some phagocytes containing haemosiderin.
4. Ultimately, after 3-4 months an old cystic infarct is formed which shows a cyst traversed by small blood vessels and has peripheral fibrillary gliosis. Small cavitory infarcts are called *lacunar infarcts* and are commonly found as a complication of systemic hypertension.

ISCHAEMIC BOWEL DISEASE (ISCHAEMIC ENTEROCOLITIS)

Ischaemic lesions of the gastrointestinal tract may occur in the small intestine and/or colon; the latter is called *ischaemic colitis* or *ischaemic enterocolitis* and is commonly

referred to as ischaemic bowel disease. In either case, the cause of ischaemia is compromised mesenteric circulation, while ischaemic effect is less likely to occur in the stomach, duodenum and rectum due to abundant collateral blood supply.

Depending upon the extent and severity of ischaemia, 3 patterns of pathologic lesions can occur:

1. *Transmural infarction*, characterised by transmural ischaemic necrosis and gangrene of the bowel.
2. *Mural infarction*, characterised by haemorrhagic gastroenteropathy (haemorrhage and necrosis). The ischaemic effect in mural infarction is limited to mucosa, submucosa and superficial muscularis, while mucosal infarction is confined to mucosal layers superficial to muscularis mucosae.
3. *Ischaemic stenosis*, due to chronic ischaemia causing fibrotic narrowing of the affected bowel.

These pathologic patterns are described below:

Transmural Infarction

Ischaemic necrosis of the full-thickness of the bowel wall is more common in the small intestine than the large intestine.

ETIOPATHOGENESIS. The common causes of transmural infarction of small bowel are as under:

i) *Mesenteric arterial thrombosis* such as due to the following:

- Atherosclerosis (most common)
- Aortic aneurysm
- Vasospasm

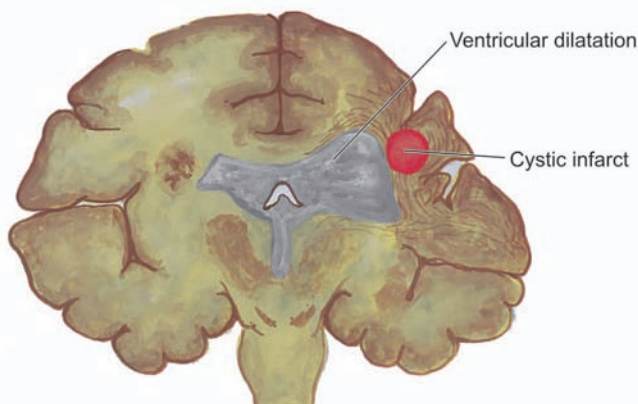


FIGURE 18.2

An old cystic infarct in the brain (coronal section). There is shrinkage of scarred area with ipsilateral ventricular dilatation.

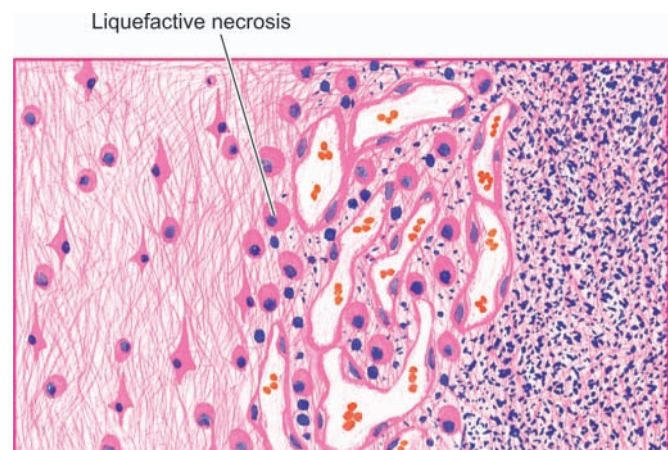


FIGURE 18.3

An anaemic infarct of a few days duration in the brain. The histologic changes are reactive astrocytosis, a few reactive macrophages and neovascularisation in the wall of the cystic lesion. The outer cortical layer is, however, intact.

- Fibromuscular hyperplasia
 - Invasion by the tumour
 - Use of oral contraceptives
 - Arteritis of various types
- ii) *Mesenteric arterial embolism* arising from the following causes:
- Mural thrombi in the heart
 - Endocarditis (infective and nonbacterial thrombotic)
 - Atherosclerotic plaques
 - Atrial myxoma
- iii) *Mesenteric venous occlusion* is less common cause of full-thickness infarction of the bowel. The causes are as under:
- Intestinal sepsis e.g. appendicitis
 - Portal venous thrombosis in cirrhosis of the liver
 - Tumour invasion
 - Use of oral contraceptives
- iv) *Miscellaneous causes*:
- Strangulated hernia
 - Torsion
 - Fibrous bands and adhesions.

PATHOLOGIC CHANGES. *Grossly*, irrespective of the underlying etiology, infarction of the bowel is haemorrhagic (red) type. A varying length of the small bowel may be affected. In the case of colonic infarction, the distribution area of superior and

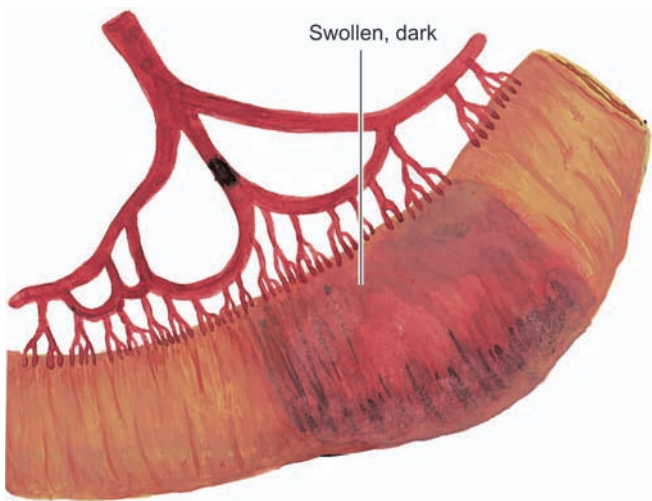


FIGURE 18.4

Haemorrhagic infarct of the small intestine. The infarcted area is swollen, dark in colour and coated with fibrinous exudate. A sharp line of demarcation separates infarcted area from the normal bowel.

inferior mesenteric arteries (i.e. *splenic flexure*) is more commonly involved. The affected areas become dark purple and markedly congested and the peritoneal surface is coated with fibrinous exudate. The wall is thickened, oedematous and haemorrhagic. The lumen is dilated and contains blood and mucus. In arterial occlusion, there is a sharp line of demarcation between the infarcted bowel and the normal intestine (Fig. 18.4), whereas in venous occlusion the infarcted area merges imperceptibly into the normal bowel.

Microscopically, there is coagulative necrosis and ulceration of the mucosa and there are extensive submucosal haemorrhages. The muscularis is less severely affected by ischaemia. Subsequently, inflammatory cell infiltration and secondary infection occur, leading to gangrene of the bowel (Fig. 18.5).

The condition is clinically characterised by 'abdominal angina' in which the patient has acute abdominal pain, nausea, vomiting, and sometimes diarrhoea. The disease is rapidly fatal, with 50-70% mortality rate.

Mural and Mucosal Infarction (Haemorrhagic Gastroenteropathy, Membranous Colitis)

Mural and mucosal infarctions are limited to superficial layers of the bowel wall, sparing the deeper layer of the muscularis and the serosa. The condition is also

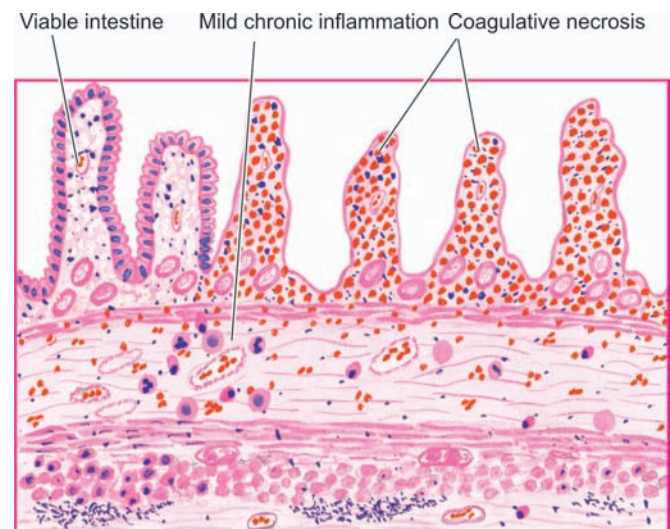


FIGURE 18.5

Infarct small intestine, microscopic appearance. The mucosa in the infarcted area shows coagulative necrosis and submucosal haemorrhages: muscularis is also partly affected. Inflammatory cell infiltration is marked at the line of demarcation between the infarcted and normal bowel.

referred to as *haemorrhagic gastroenteropathy*, and in the case of colon as *membranous colitis*.

ETIOPATHOGENESIS. Haemorrhagic gastroenteropathy results from conditions causing non-occlusive hypoperfusion (compared from transmural infarction which occurs from occlusive causes). These are as under:

- Shock
- Cardiac failure
- Infections
- Intake of drugs causing vasoconstriction e.g. digitalis, norepinephrine.

PATHOLOGIC CHANGES. *Grossly*, the lesions affect variable length of the bowel. The affected segment of the bowel is red or purple but without haemorrhage and exudation on the serosal surface. The mucosa is oedematous at places, sloughed and ulcerated at other places. The lumen contains haemorrhagic fluid.

Microscopically, there is patchy ischaemic necrosis of mucosa, vascular congestion, haemorrhages and inflammatory cell infiltrate. The changes may extend into superficial muscularis but deeper layer of muscularis and serosa are spared. Secondary bacterial infection may supervene resulting in pseudo-membranous enterocolitis.

Clinically, as in transmural infarction, the features of abdominal pain, nausea, vomiting and diarrhoea are present, but the changes are reversible and curable. With adequate therapy, normal morphology is completely restored in superficial lesions, while deeper lesions may heal by fibrosis leading to stricture formation.

Ischaemic Stenosis

Although this condition affects primarily colon in the region of splenic flexure, it is described here due to its apparent pathogenetic relationship with ischaemic injury. The disease has 2 phases:

- i) **Ischaemic colitis**—characterised by acute colonic necrosis as described above.
- ii) **Ischaemic stricture**—occurring following segmental inflammation of the colon.

Grossly, there is fusiform or saccular external appearance. There is patchy and longitudinal mucosal ulceration.

Microscopically, the ulcerated areas of the mucosa show granulation tissue. The submucosa is characteristically thickened due to inflammation and fibrosis. The muscularis may also show inflammatory

changes and patchy replacement by fibrosis. The blood vessels may show atheromatous emboli, organising thrombi and endarteritis obliterans.

INFARCT LUNG

Embolism of the pulmonary arteries may produce pulmonary infarction, though not always. This is because lungs receive blood supply from bronchial arteries as well, and thus occlusion of pulmonary artery ordinarily does not produce infarcts. However, it may occur in patients who have inadequate circulation such as in chronic lung diseases and congestive heart failure.

Grossly, the pulmonary infarcts are classically wedge-shaped with base on the pleura, haemorrhagic, variable in size, and most often in the lower lobes. Fibrinous pleuritis usually covers the area of infarct. Cut surface is dark purple and may show the blocked vessel near the apex of the infarcted area. Old organised and healed pulmonary infarcts appear as retracted fibrous scars.

Microscopically, the characteristic histologic feature is coagulative necrosis of the alveolar walls. Initially, there is infiltration by neutrophils and intense alveolar capillary congestion, but later their place is taken by haemosiderin, phagocytes and granulation tissue (**COLOUR PLATE II: CL 8**).

INFARCT KIDNEY

Renal infarction is common, found in upto 5% of autopsies. Majority of them are caused by thromboemboli, most commonly originating from the heart such as in mural thrombi in the left atrium, myocardial infarction, vegetative endocarditis and aortic aneurysm. Less commonly, renal infarcts may occur due to advanced renal artery atherosclerosis, arteritis and sickle cell anaemia.

Grossly, renal infarcts are often multiple and may be bilateral. Characteristically, they are pale or anaemic and wedge-shaped with base resting under the capsule and apex pointing towards the medulla. Generally, a narrow rim of preserved renal tissue under the capsule is spared because it draws its blood supply from the capsular vessels. Cut surface of renal infarct in the first 2 to 3 days is red and congested but by 4th day the centre becomes pale yellow. At the end of one week, the infarct is typically anaemic and depressed below the surface of the kidney (Fig. 18.6).

Microscopically, the affected area shows characteristic coagulative necrosis of renal parenchyma i.e.

Pale infarct

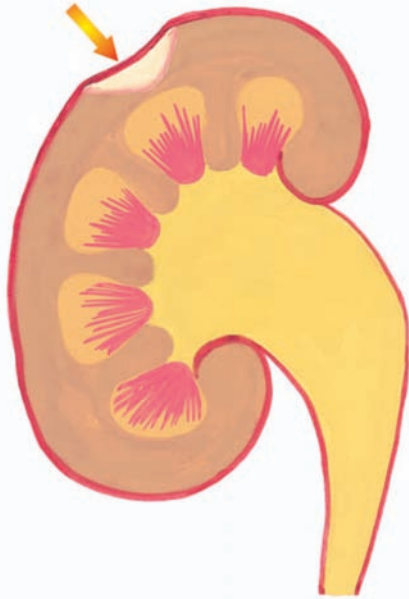


FIGURE 18.6

Infarct kidney, gross appearance. The wedge-shaped infarct is slightly depressed on the surface. The apex lies internally and wide base is on the surface. The central area is pale while the margin is haemorrhagic.

there are ghosts of renal tubules and glomeruli without intact nuclei and cytoplasmic content. The margin of the infarct shows inflammatory reaction—initially acute but later macrophages and fibrous tissue predominate (Fig. 18.7) (COLOUR PLATE II: CL 7).

INFARCT SPLEEN

Spleen is one of the common sites for infarction. Splenic infarction results from occlusion of the splenic artery or its branches. Occlusion is caused most commonly by thromboemboli arising in the heart (e.g. in mural thrombi in the left atrium, vegetative endocarditis, myocardial infarction), and less frequently by obstruction of microcirculation (e.g. in myeloproliferative diseases, sickle cell anaemia, arteritis, Hodgkin's disease, bacterial infections).

Grossly, splenic infarcts are often multiple. They are characteristically pale or anaemic and wedge-shaped with their base at the periphery and apex pointing towards hilum.

Microscopically, the features are similar to those found in anaemic infarcts in kidney. Coagulative necrosis and inflammatory reaction are seen. Later,

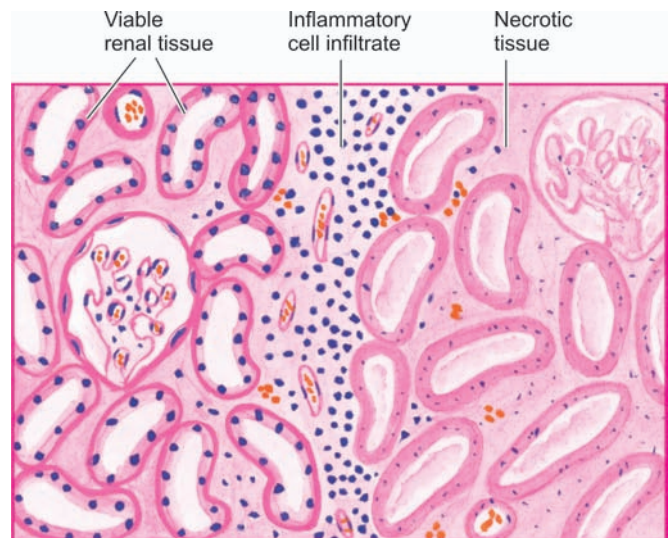


FIGURE 18.7

Microscopic appearance of renal infarct. Renal tubules and glomeruli show typical coagulative necrosis i.e. intact outlines of necrosed cells. There is dense acute inflammatory infiltrate at the periphery of the infarct.

the necrotic tissue is replaced by shrunken fibrous scar.

INFARCT LIVER

Just as in lungs, infarcts in the liver are uncommon due to dual blood supply—from portal vein and from hepatic artery. Obstruction of the portal vein is usually secondary to other diseases such as hepatic cirrhosis, intravenous invasion of primary carcinoma of the liver, carcinoma of the pancreas and pylephlebitis. Occlusion of portal vein or its branches generally does not produce ischaemic infarction but instead reduced blood supply to hepatic parenchyma causes non-ischaemic infarct called *infarct of Zahn*. Obstruction of the hepatic artery or its branches, on the other hand, caused by arteritis, arteriosclerosis, bland or septic emboli, results in ischaemic infarcts of the liver.

Grossly, ischaemic infarcts of the liver are usually anaemic but sometimes may be haemorrhagic due to stuffing of the site by blood from the portal vein. Infarcts of Zahn (non-ischaemic infarcts) produce sharply defined red-blue area in liver parenchyma. **Microscopically**, ischaemic infarcts show characteristics of pale or anaemic infarcts as in kidney or spleen. Infarcts of Zahn occurring due to reduced portal blood flow result in atrophy of hepatocytes and dilatation of sinusoids.



Growth Disorders and Neoplasia

SECTION CONTENTS

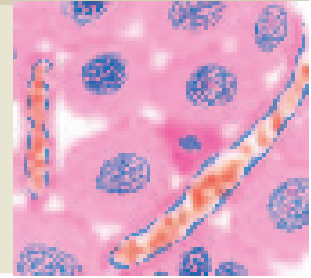
CHAPTER 19: Cellular Adaptations

CHAPTER 20: General Aspects of Neoplasia

CHAPTER 21: Etiology and Pathogenesis of Neoplasia

CHAPTER 22: Clinical Aspects of Neoplasia

CHAPTER 23: Common Specific Tumours



19

Cellular Adaptations

For the sake of survival on exposure to stress, the cells make adjustments with the changes in their environment (i.e. adapt) to the physiologic needs (*physiologic adaptation*) and to non-lethal pathologic injury (*pathologic adaptation*). Broadly speaking, such physiologic and pathologic adaptations occur by following processes (Fig. 19.1):

- Decreasing or increasing their size i.e. *atrophy* and *hypertrophy* respectively, or by increasing their number i.e. *hyperplasia*.
- By changing the pathway of phenotypic differentiation of cells i.e. *metaplasia* and *dysplasia*.

In general, the adaptive responses are reversible on withdrawal of stimulus. However, if the irritant stimulus persists for long time, the cell may not be able to survive and may either die or progress further e.g. cell death in sustained atrophy, progression of dysplasia into carcinoma *in situ*. Thus, the concept of evolution 'survival of the fittest' holds true for adaptation as 'survival of the adaptable'.

Various mechanisms which may be involved in adaptive cellular responses include:

- Altered cell surface receptor binding.
- Alterations in signal for protein synthesis.
- Synthesis of new proteins by the target cell such as heat-shock proteins (HSPs).

Common forms of cellular adaptive responses along with examples of physiologic and pathologic adaptations are briefly discussed below.

ATROPHY

Reduction of the number and size of parenchymal cells of an organ or its parts which was once normal is called atrophy (c.f. *hypoplasia* which is the term used for developmentally small size, and *aplasia* for extreme failure of development so that only rudimentary tissue is present).

CAUSES. Atrophy may occur from physiologic or pathologic causes.

A. Physiologic atrophy. Atrophy is a normal process of aging in some tissues which could be due to loss of endocrine stimulation or arteriosclerosis. For example:

- i) Atrophy of lymphoid tissue in lymph nodes, appendix and thymus.
- ii) Atrophy of gonads after menopause.
- iii) Atrophy of brain.

B. Pathologic atrophy. The causes are as under:

1. *Starvation atrophy.* In starvation, there is first depletion of carbohydrate and fat stores followed by protein catabolism. There is general weakness, emaciation and

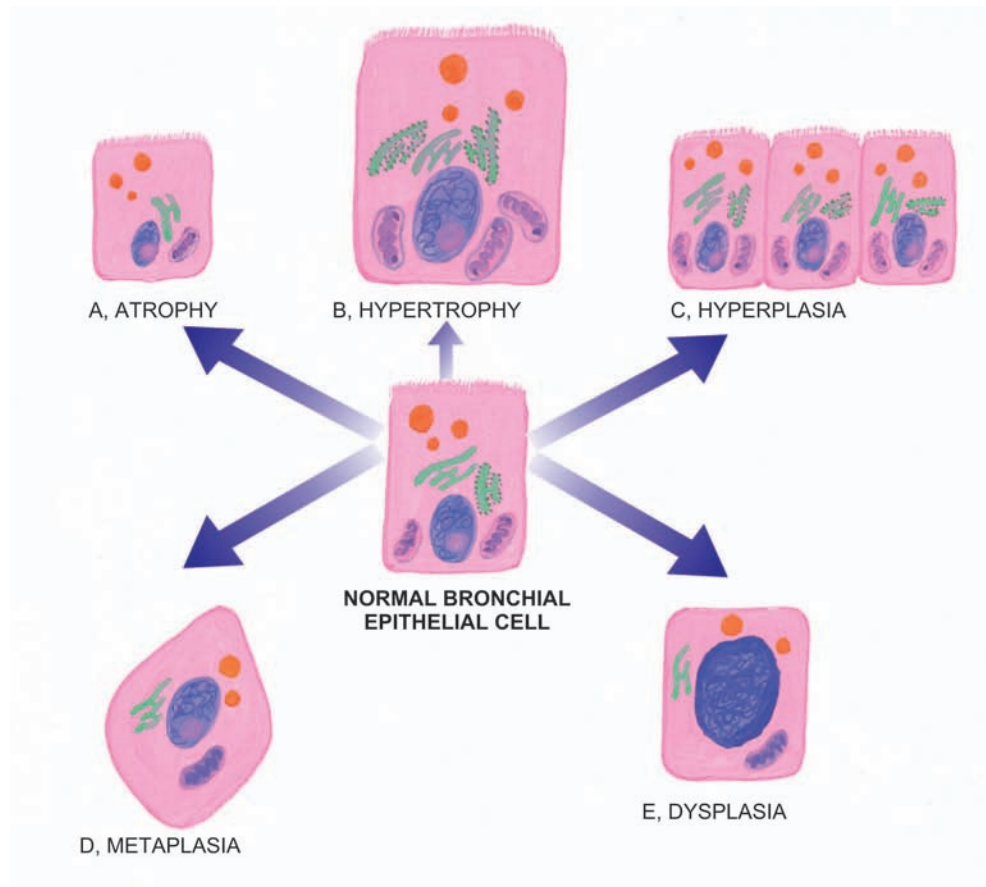


FIGURE 19.1

Adaptive disorders of growth.

anaemia referred to as cachexia seen in cancer and severely-ill patients.

2. *Ischaemic atrophy.* Gradual diminution of blood supply due to atherosclerosis may result in shrinkage of the affected organ e.g.

- i) Small atrophic kidney in atherosclerosis of renal artery.
- ii) Atrophy of brain in cerebral atherosclerosis.

3. *Disuse atrophy.* Prolonged diminished functional activity is associated with disuse atrophy of the organ e.g.

- i) Wasting of muscles of limb immobilised in cast.
- ii) Atrophy of the pancreas in obstruction of pancreatic duct.

4. *Neuropathic atrophy.* Interruption in nerve supply leads to wasting of muscles e.g.

- i) Poliomyelitis
- ii) Motor neuron disease
- iii) Nerve section.

5. *Endocrine atrophy.* Loss of endocrine regulatory mechanism results in reduced metabolic activity of tissues and hence atrophy e.g.

- i) Hypopituitarism may lead to atrophy of thyroid, adrenal and gonads.
- ii) Hypothyroidism may cause atrophy of the skin and its adnexal structures.

6. *Pressure atrophy.* Prolonged pressure from benign tumours or cyst or aneurysm may cause compression and atrophy of the tissues e.g.

- i) Erosion of spine by tumour in nerve root.
- ii) Erosion of skull by meningioma arising from pia-arachnoid.
- iii) Erosion of sternum by aneurysm of arch of aorta.

7. *Idiopathic atrophy.* There are some examples of atrophy where no obvious cause is present e.g.

- i) Myopathies.
- ii) Testicular atrophy.

PATHOLOGIC CHANGES. Irrespective of the underlying cause for atrophy, the pathologic changes are similar. The organ is small, often shrunk. The cells become smaller in size but are not dead cells (Fig. 19.1, A). Shrinkage in cell size is due to reduction in cell organelles, chiefly mitochondria, myofilaments and endoplasmic reticulum. There is often increase in the number of autophagic vacuoles containing cell debris. These autophagic vacuoles may persist to form 'residual bodies' in the cell cytoplasm e.g. lipofuscin pigment granules in brown atrophy (page 50).

HYPERTROPHY

Hypertrophy is an increase in the size of parenchymal cells resulting in enlargement of the organ or tissue, without any change in the number of cells.

CAUSES. Hypertrophy may be physiologic or pathologic. In both cases, it is caused either by increased functional demand or by hormonal stimulation. Hypertrophy without accompanying hyperplasia affects mainly muscles. In non-dividing cells too, only hypertrophy occurs.

A. Physiologic hypertrophy. Enlarged size of the uterus in pregnancy is an excellent example of physiologic hypertrophy as well as hyperplasia.

B. Pathologic hypertrophy. Examples of certain diseases associated with hypertrophy are as under:

1. *Hypertrophy of cardiac muscle* may occur in a number of cardiovascular diseases. A few examples producing left ventricular hypertrophy are:
 - i) Systemic hypertension
 - ii) Aortic valve disease (stenosis and insufficiency)
 - iii) Mitral insufficiency
2. *Hypertrophy of smooth muscle* e.g.
 - i) Cardiac achalasia (in oesophagus)
 - ii) Pyloric stenosis (in stomach)
 - iii) Intestinal strictures
 - iv) Muscular arteries in hypertension.
3. *Hypertrophy of skeletal muscle* e.g. hypertrophied muscles in athletes and manual labourers.
4. *Compensatory hypertrophy* may occur in an organ when the contralateral organ is removed e.g.
 - i) Following nephrectomy on one side in a young patient, there is compensatory hypertrophy as well as hyperplasia of the nephrons of the other kidney.
 - ii) Adrenal hyperplasia following removal of one adrenal gland.

PATHOLOGIC CHANGES. The affected organ is enlarged and heavy. For example a hypertrophied heart of a patient with systemic hypertension may weigh 700-800 g as compared to average normal adult weight of 350 g. There is enlargement of muscle fibres as well as of nuclei (Fig. 19.1,B). At ultrastructural level, there is increased synthesis of DNA and RNA, increased protein synthesis and increased number of organelles like mitochondria, endoplasmic reticulum and myofibrils.

HYPERPLASIA

Hyperplasia is an increase in the number of parenchymal cells resulting in enlargement of the organ or tissue. Quite often, both hyperplasia and hypertrophy occur together. Hyperplasia occurs due to increased recruitment of cells from G_0 (resting) phase of the cell cycle to undergo mitosis, when stimulated. All body cells do not possess hyperplastic growth potential. Labile cells (e.g. epithelial cells of the skin and mucous membranes, cells of the bone marrow and lymph nodes) and stable cells (e.g. parenchymal cells of the liver, pancreas, kidney, adrenal, and thyroid) can undergo hyperplasia, while permanent cells (e.g. neurons, cardiac and skeletal muscle) have little or no capacity for regenerative hyperplastic growth. Neoplasia differs from hyperplasia in having hyperplastic growth with loss of growth-regulatory mechanism due to change in genetic composition of the cell. Hyperplasia, on the other hand, persists so long as stimulus is present.

CAUSES. As with other non-neoplastic disorders of growth, hyperplasia has also been divided into physiologic and pathologic.

A. Physiologic hyperplasia. The two most common types are as follows:

1. *Hormonal hyperplasia* i.e. hyperplasia occurring under the influence of hormonal stimulation e.g.
 - i) Hyperplasia of female breast at puberty, during pregnancy and lactation.
 - ii) Hyperplasia of pregnant uterus.
 - iii) Proliferative activity of normal endometrium after a normal menstrual cycle.
 - iv) Prostatic hyperplasia in old age.
2. *Compensatory hyperplasia* i.e. hyperplasia occurring following removal of part of an organ or a contralateral organ in paired organ e.g.
 - i) Regeneration of the liver following partial hepatectomy
 - ii) Regeneration of epidermis after skin abrasion
 - iii) Following nephrectomy on one side, there is hyperplasia of nephrons of the other kidney.

B. Pathologic hyperplasia. Most examples of pathologic hyperplasia are due to excessive stimulation of hormones or growth factors e.g.

- i) Endometrial hyperplasia following oestrogen excess.
- ii) In wound healing, there is formation of granulation tissue due to proliferation of fibroblasts and endothelial cells.
- iii) Formation of skin warts from hyperplasia of epidermis due to human papilloma virus.
- iv) Pseudocarcinomatous hyperplasia of the skin.
- v) Intraductal epithelial hyperplasia in the breast in fibrocystic breast disease.

PATHOLOGIC CHANGES. There is enlargement of the affected organ or tissue and increase in the number of cells (Fig. 19.1,C). This is due to increased rate of DNA synthesis and hence increased mitoses of the cells.

METAPLASIA

Metaplasia (*meta* = transformation, *plasia* = growth) is defined as a reversible change of one type of epithelial or mesenchymal adult cells to another type of adult epithelial or mesenchymal cells, usually in response to abnormal stimuli, and often *reverts back to normal on removal of stimulus* (Fig. 19.1,D). However, if the stimulus persists for a long time, epithelial metaplasia may transform into cancer.

Metaplasia is broadly divided into 2 types: epithelial and mesenchymal.

A. EPITHELIAL METAPLASIA. This is the more common type. The metaplastic change may be patchy or diffuse and usually results in replacement by stronger but less well-specialised epithelium. However, the metaplastic epithelium being less well-specialised such as squamous type, results in deprivation of protective mucus secretion and hence more prone to infection. Some common types of epithelial metaplasia are as under:

1. **Squamous metaplasia.** Various types of epithelium are capable of undergoing squamous metaplastic change due to chronic irritation that may be mechanical, chemical or infective in origin. Some common examples of squamous metaplasia are seen at following sites:
 - i) In *bronchus* (normally lined by pseudostratified columnar ciliated epithelium) in chronic smokers.
 - ii) In *uterine endocervix* (normally lined by simple columnar epithelium) in prolapse of the uterus and in old age (Fig. 19.2, A and B) (**COLOUR PLATE VI: CL 21**).
 - iii) In *gallbladder* (normally lined by simple columnar epithelium) in chronic cholecystitis with cholelithiasis.

- iv) In *prostate* (ducts normally lined by simple columnar epithelium) in chronic prostatitis and oestrogen therapy.
- v) In *renal pelvis* and *urinary bladder* (normally lined by transitional epithelium) in chronic infection and stones.
- vi) In *vitamin A deficiency*, apart from xerophthalmia, there is squamous metaplasia in the nose, bronchi, urinary tract, lacrimal and salivary glands.

2. **Columnar metaplasia.** There are some conditions in which there is transformation to columnar epithelium. For example:

- i) Intestinal metaplasia in healed chronic gastric ulcer.
- ii) Conversion of pseudostratified columnar epithelium in chronic bronchitis and bronchiectasis to columnar type.
- iii) In cervical erosion (congenital and adult type), there is variable area of endocervical glandular mucosa everted into the vagina.

B. MESENCHYMAL METAPLASIA. Less often, there is transformation of one adult type of mesenchymal tissue to another. The examples are as under:

1. **Osseous metaplasia.** Osseous metaplasia is formation of bone in fibrous tissue, cartilage and myxoid tissue. Examples of osseous metaplasia are as under:

- i) In arterial wall in old age (Mönckeberg's medial calcific sclerosis)
- ii) In soft tissues in myositis ossificans
- iii) In cartilage of larynx and bronchi in elderly people
- iv) In scar of chronic inflammation of prolonged duration
- v) In the fibrous stroma of tumour.

2. **Cartilaginous metaplasia.** In healing of fractures, cartilaginous metaplasia may occur where there is undue mobility.

DYSPLASIA

Dysplasia means 'disordered cellular development', often accompanied with metaplasia and hyperplasia; it is therefore also referred to as *atypical hyperplasia*. Dysplasia occurs most often in epithelial cells. Epithelial dysplasia is characterised by cellular proliferation and cytologic changes. These changes include:

1. Increased number of layers of epithelial cells
2. Disorderly arrangement of cells from basal layer to the surface layer
3. Loss of basal polarity i.e. nuclei lying away from basement membrane
4. Cellular and nuclear pleomorphism
5. Increased nucleocytoplasmic ratio
6. Nuclear hyperchromatism
7. Increased mitotic activity.

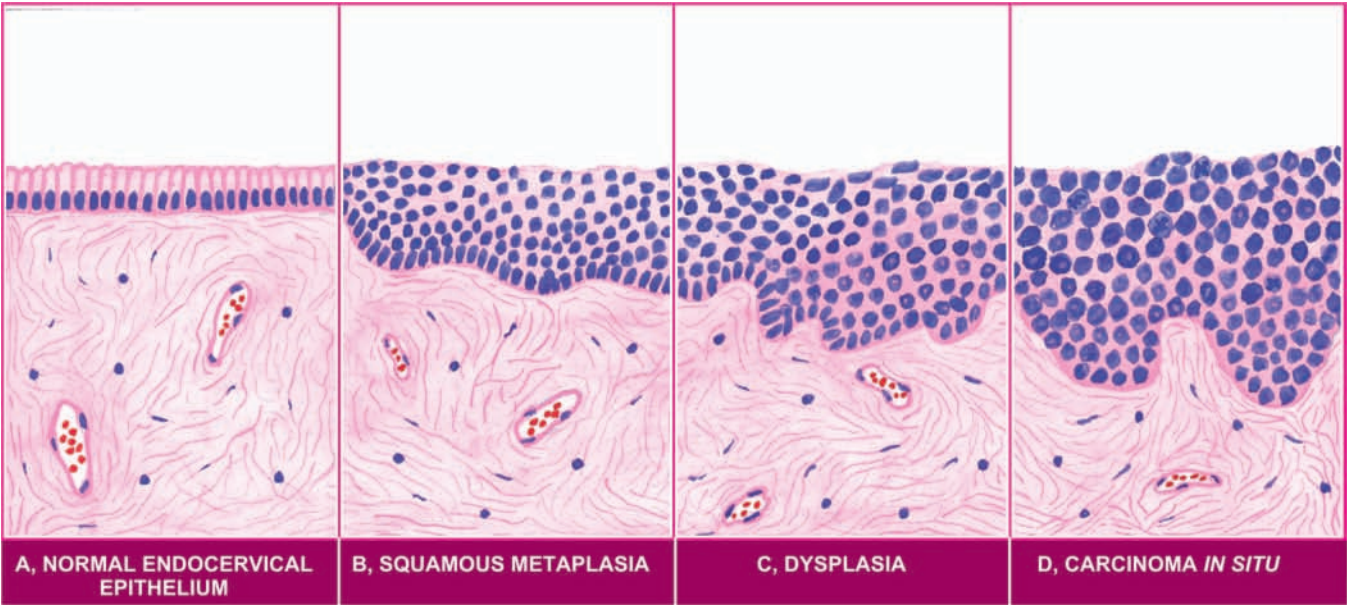


FIGURE 19.2

Schematic diagram showing sequential changes in uterine cervix from normal epithelium to development of carcinoma *in situ*. A, Normal mucus-secreting endocervical epithelium. B, Squamous metaplasia. C, Dysplastic change. D, Carcinoma *in situ*.

The two most common examples of dysplastic changes are the *uterine cervix* (Fig. 19.2, C) and *respiratory tract*.

Dysplastic changes often occur due to chronic irritation or prolonged inflammation. On removal of the inciting stimulus, the changes may disappear. In a proportion of cases, however, dysplasia progresses into carcinoma *in situ* (cancer confined to layers superficial to basement membrane) (Fig. 19.2, D) or invasive cancer (COLOUR PLATE VI: CL 22).

The differences between dysplasia and metaplasia are contrasted in Table 19.1.

CELLULAR AGING

Old age is a concept of longevity in human beings. The consequences of aging appear after reproductive age. However, aging is distinct from mortality and disease although aged individuals are more vulnerable to disease.

The average age of death of primitive man was barely 20-25 years compared to life-expectancy now which is approaching 80 years, survival being longer in women than men (3:2). About a century ago, the main causes of death were accidents and infections. But now with greater safety and sanitation, the mortality in the

TABLE 19.1: Differences between Metaplasia and Dysplasia.

FEATURE	METAPLASIA	DYSPLASIA
i) Definition	Change of one type of epithelial or mesenchymal cell to another type of adult epithelial or mesenchymal cell	Disordered cellular development, may be accompanied with hyperplasia or metaplasia
ii) Types	Epithelial (squamous, columnar) and mesenchymal (osseous, cartilaginous)	Epithelial only
iii) Tissues affected	Most commonly affects bronchial mucosa, uterine endocervix; others mesenchymal tissues (cartilage, arteries)	Uterine cervix, bronchial mucosa
iv) Cellular changes	Mature cellular development	Disordered cellular development (pleomorphism, nuclear hyperchromasia, mitosis, loss of polarity)
v) Natural history	Reversible on withdrawal of stimulus	May regress on removal of inciting stimulus, or may progress to higher grades of dysplasia or carcinoma <i>in situ</i>

middle years has sufficiently declined. However, the maximum human lifespan has remained stable at about 110 years. Higher life expectancy in women is not due to difference in the response of somatic cells of the two sexes but higher mortality rate in men is attributed to violent causes and greater susceptibility to cardiovascular disease, cancer, cirrhosis and respiratory diseases, for which cigarette smoking and alcohol consumption are important contributory factors.

In general, the life expectancy of an individual depends upon the following factors:

1. *Intrinsic genetic process* i.e the genes controlling response to endogenous and exogenous factors initiating apoptosis in senility
2. *Environmental factors* e.g. consumption and inhalation of harmful substances, diet, role of antioxidants etc.
3. *Lifestyle of the individual* such as diseases due to alcoholism (e.g. cirrhosis, hepatocellular carcinoma), smoking (e.g. bronchogenic carcinoma and other respiratory diseases), drug addiction.
4. *Age-related diseases* e.g. atherosclerosis and ischaemic heart disease, diabetes mellitus, hypertension, osteoporosis, Alzheimer's disease, Parkinson's disease etc.

CELLULAR BASIS

With age, structural and functional changes occur in different organs and systems of the human body. Although no definitive biologic basis of aging is established, most acceptable theory is the functional decline of non-dividing cells such as neurons and myocytes. The following hypotheses based on investigations explain the cellular basis of aging:

1. Experimental cellular senescence. By *in vitro* studies of tissue culture, it has been observed that cultured human fibroblasts replicate for upto 50 population doublings and then the culture dies out. It means that *in vitro* there is reduced functional capacity to proliferate with age. Studies have shown that there is either loss of chromosome 1 or deletion of its long arm (1q). Alternatively it has been observed that with every cell division there is progressive shortening of *telomere* present at the tips of chromosomes which in normal cell is repaired by the presence of RNA enzyme, *telomerase*. However, due to aging, due to inadequate presence of telomerase enzyme, lost telomere is not repaired resulting in interference in viability of cell.

2. Genetic control in invertebrates. Clock (*clk*) genes responsible for controlling the rate and time of aging have been identified in lower invertebrates e.g. *clk-1* gene mutation in the metazoa, *Caenorhabditis elegans*,

results in prolonging the lifespan of the worm and slowing of some metabolic functions.

3. Diseases of accelerated aging. Aging is under genetic control in human beings supported by the observation of high concordance in lifespan of identical twins. A heritable condition associated with signs of accelerated aging process is termed *progeria* and is characterised by baldness, cataracts, and coronary artery disease. Another example is Werner syndrome, a rare autosomal recessive disease, characterised by similar features of premature aging, atherosclerosis and risk for development of various cancers.

4. Oxidative stress hypothesis. Currently, it is believed that aging is partly caused by progressive and reversible molecular oxidative damage due to persistent oxidative stress on the human cells. In normal cells, very small amount (3%) of total oxygen consumption by the cell is converted into reactive oxygen species. The rate of generation of reactive oxygen species is directly correlated with metabolic rate of the organisms. With aging, there is low metabolic rate with generation of toxic oxygen radicals which fail to get eliminated causing their accumulation and cell damage. The underlying mechanism appears to be oxidative damage to mitochondria. The role of antioxidant in retarding the oxidant damage has been reported in some studies.

ORGAN CHANGES IN AGING

Although all organs start showing deterioration with aging, following organs show evident morphologic and functional changes:

1. *Cardiovascular system:* Atherosclerosis, arteriosclerosis with calcification, Mönckeberg's medial calcification, brown atrophy of heart, loss of elastic tissue from aorta and major arterial trunks causing their dilatation.
2. *Nervous system:* Atrophy of gyri and sulci, Alzheimer's disease, Parkinson's disease.
3. *Musculoskeletal system:* Degenerative bone diseases, frequent fractures due to loss of bone density, age related muscular degeneration.
4. *Eyes:* Deterioration of vision due to cataract and vascular changes in retina.
5. *Hearing:* Disability in hearing due to senility is related to otosclerosis.
6. *Immune system:* reduced IgG response to antigens, frequent and severe infections.
7. *Skin:* Laxity of skin due to loss of elastic tissue.
8. *Cancers:* As discussed in next chapter, 80% of cancers occur in the age range of 50 and 80 years.



NOMENCLATURE AND CLASSIFICATION

INTRODUCTION. The term 'neoplasia' means new growth; the new growth produced is called 'neoplasm' or 'tumour'. However, all 'new growths' are not neoplasms since examples of new growth of tissues and cells also exist in the processes of embryogenesis, regeneration and repair, hyperplasia and hormonal stimulation. The proliferation and maturation of cells in normal adults is controlled as a result of which some cells proliferate throughout life (labile cells), some have limited proliferation (stable cells), while others do not replicate (permanent cells). On the other hand, neoplastic cells lose control and regulation of replication and form an abnormal mass of tissue.

Therefore, satisfactory definition of a neoplasm or tumour is 'a mass of tissue formed as a result of abnormal, excessive, uncoordinated, autonomous and purposeless proliferation of cells'. The branch of science dealing with the study of neoplasms or tumours is called oncology (*oncos*=tumour, *logos*=study). Neoplasms may be 'benign' when they are slow-growing and localised without causing much difficulty to the host, or 'malignant' when they proliferate rapidly, spread throughout the body and may eventually cause death of the host. The common term used for all malignant tumours is cancer. Hippocrates (460-377 BC) coined the term *karkinos* for cancer of the breast. The word 'cancer' means crab, thus reflecting the true character of cancer since 'it sticks to the part stubbornly like a crab'.

All tumours, benign as well as malignant, have 2 basic components:

■ '*Parenchyma*' comprised by proliferating tumour cells; parenchyma determines the nature and evolution of the tumour.

■ '*Supportive stroma*' composed of fibrous connective tissue and blood vessels; it provides the framework on which the parenchymal tumour cells grow.

The tumours derive their nomenclature on the basis of the parenchymal component comprising them. The suffix '-oma' is added to denote benign tumours. Malignant tumours of epithelial origin are called *carcinomas*, while malignant mesenchymal tumours are named *sarcomas* (*sarcos* = fleshy). However, some cancers are

composed of highly undifferentiated cells and are referred to as *undifferentiated malignant tumours*.

Although, this broad generalisation regarding nomenclature of tumours usually holds true in majority of instances, some examples contrary to this concept are: *melanoma* for carcinoma of the melanocytes, *hepatoma* for carcinoma of the hepatocytes, *lymphoma* for malignant tumour of the lymphoid tissue, and *seminoma* for malignant tumour of the testis. *Leukaemia* is the term used for cancer of blood forming cells.

SPECIAL CATEGORIES OF TUMOURS. Following categories of tumours are examples which defy the generalisation in nomenclature given above:

1. Mixed tumours. When two types of tumours are combined in the same tumour, it is called a mixed tumour. For example:

i) *Adenosquamous carcinoma* is the combination of adenocarcinoma and squamous cell carcinoma in the endometrium.

ii) *Adenoacanthoma* is the mixture of adenocarcinoma and benign squamous elements in the endometrium.

iii) *Carcinosarcoma* is the rare combination of malignant tumour of the epithelium (carcinoma) and of mesenchymal tissue (sarcoma) such as in thyroid.

iv) *Collision tumour* is the term used for morphologically two different cancers in the same organ which do not mix with each other.

v) *Mixed tumour of the salivary gland* (or pleomorphic adenoma) is the term used for benign tumour having combination of both epithelial and mesenchymal tissue elements.

2. Teratomas. These tumours are made up of a mixture of various tissue types arising from totipotent cells derived from the three germ cell layers—ectoderm, mesoderm and endoderm. Most common sites for teratomas are ovaries and testis (*gonadal teratomas*). But they occur at *extra-gonadal sites* as well, mainly in the midline of the body such as in the head and neck region, mediastinum, retroperitoneum, sacrococcygeal region etc. Teratomas may be *benign or mature* (most of the ovarian teratomas) or *malignant or immature* (most of the testicular teratomas).

3. Blastomas. Blastomas or embryomas are a group of malignant tumours which arise from embryonal or

partially differentiated cells which would normally form blastoma of the organs and tissue during embryogenesis. These tumours occur more frequently in infants and children (under 5 years of age) and include some examples of tumours in this age group: neuroblastoma, nephroblastoma (Wilms' tumour), hepatoblastoma, retinoblastoma, medulloblastoma, pulmonary blastoma.

4. Hamartoma. Hamartoma is benign tumour which is made of mature but disorganised cells of tissues indigenous to the particular organ e.g. hamartoma of the lung consists of mature cartilage, mature smooth muscle and epithelium. Thus, all mature differentiated tissue elements which comprise the bronchus are present in it but are jumbled up as a mass.

5. Choristoma. Choristoma is the name given to the ectopic islands of normal tissue. Thus, choristoma is heterotopia but is not a true tumour, though it sounds like one.

CLASSIFICATION. The currently used classification of tumours is based on the histogenesis (i.e. cell of origin) and on the anticipated behaviour (Table 20.1). However, it must be mentioned here that the classification described here is only a summary. Detailed classifications of benign and malignant tumours pertaining to different tissues and body systems alongwith morphologic features of specific tumours appear in the specific chapters of Systemic Pathology later.

CHARACTERISTICS OF TUMOURS

Majority of neoplasms can be categorised clinically and morphologically into benign and malignant on the basis of certain characteristics listed below. However, there are exceptions. A small proportion of tumours have some features suggesting innocent growth while other features point towards a more ominous behaviour. Therefore, it must be borne in mind before describing characteristics of neoplasm that there is a wide variation in the degree of deviation from the normal in all the tumours.

The characteristics of tumours are described under the following headings:

- I. Rate of growth
- II. Clinical and gross features
- III. Microscopic features
- IV. Local invasion (Direct spread)
- V. Metastasis (Distant spread)

Based on these characteristics, contrasting features of benign and malignant tumours are summarised in Table 20.2 and illustrated in Fig. 20.1.

I. RATE OF GROWTH

The tumour cells generally proliferate more rapidly than the normal cells. In general, benign tumours grow slowly and malignant tumours rapidly. However, there are exceptions to this generalisation. The rate at which the tumour enlarges depends upon 2 main factors:

1. Rate of division and destruction of tumour cells
2. Degree of differentiation of the tumour.

1. Rate of division and destruction of tumour cells.

In general, malignant tumour cells have increased mitotic rate and slower death rate i.e. the cancer cells do not follow normal controls in cell cycle and are immortal. If the rate of cell division is high, it is likely that tumour cells in the centre of the tumour do not receive adequate nourishment and undergo ischaemic necrosis. While dead tumour cells appear as 'apoptotic figures' (page 38), the dividing cells of tumours are seen as normal and abnormal 'mitotic figures' (discussed later).

2. Degree of differentiation. Secondly, the rate of growth of malignant tumour is directly proportionate to the degree of differentiation. Poorly differentiated tumours show aggressive growth pattern as compared to better differentiated tumours. Some tumours, after a period of slow growth, may suddenly show spurt in their growth due to development of an aggressive clone of malignant cells. On the other hand, some tumours may cease to grow after sometime. Rarely, a malignant tumour may disappear spontaneously from the primary site, possibly due to necrosis caused by good host immune attack, only to reappear as secondaries elsewhere in the body e.g. choriocarcinoma, malignant melanoma.

The regulation of tumour growth is under the control of growth factors secreted by the tumour cells. Out of various growth factors, important ones modulating tumour biology are listed below and discussed later:

- i) Epidermal growth factor (EGF)
- ii) Fibroblast growth factor (FGF)
- iii) Platelet-derived growth factor (PDGF)
- iv) Colony stimulating factor (CSF)
- v) Transforming growth factors- β (TGF- β)
- vi) Interleukins (IL).

II. CLINICAL AND GROSS FEATURES

Clinically, benign tumours are generally slow growing, and depending upon the location, may remain asymptomatic (e.g. subcutaneous lipoma), or may produce serious symptoms (e.g. meningioma in the

TABLE 20.1: Classification of Tumours.

TISSUE OF ORIGIN	BENIGN	MALIGNANT
I. TUMOURS OF ONE PARENCHYMAL CELL TYPE		
A. Epithelial Tumours		
1. Squamous epithelium	Squamous cell papilloma	Squamous cell (Epidermoid) carcinoma
2. Transitional epithelium	Transitional cell papilloma	Transitional cell carcinoma
3. Glandular epithelium	Adenoma	Adenocarcinoma
4. Basal cell layer skin	—	Basal cell carcinoma
5. Neuroectoderm	Naevus	Melanoma (melanocarcinoma)
6. Hepatocytes	Liver cell adenoma	Hepatoma (hepatocellular carcinoma)
7. Placenta (Chorionic epithelium)	Hydatidiform mole	Choriocarcinoma
B. Non-epithelial (Mesenchymal) tumours		
1. Adipose tissue	Lipoma	Liposarcoma
2. Adult fibrous tissue	Fibroma	Fibrosarcoma
3. Embryonic fibrous tissue	Myxoma	Myxosarcoma
4. Cartilage	Chondroma	Chondrosarcoma
5. Bone	Osteoma	Osteosarcoma
6. Synovium	Benign synovioma	Synovial sarcoma
7. Smooth muscle	Leiomyoma	Leiomyosarcoma
8. Skeletal muscle	Rhabdomyoma	Rhabdomyosarcoma
9. Mesothelium	—	Mesothelioma
10. Blood vessels	Haemangioma	Angiosarcoma
11. Lymph vessels	Lymphangioma	Lymphangiosarcoma
12. Glomus	Glomus tumour	—
13. Meninges	Meningioma	Invasive meningioma
14. Haematopoietic cells	—	Leukaemias
15. Lymphoid tissue	Pseudolymphoma	Malignant lymphomas
16. Nerve sheath	Neurilemmoma, Neurofibroma	Neurogenic sarcoma
17. Nerve cells	Ganglioneuroma	Neuroblastoma
II. MIXED TUMOURS		
Salivary glands	Pleomorphic adenoma (mixed salivary tumour)	Malignant mixed salivary tumour
III. TUMOURS OF MORE THAN ONE GERM CELL LAYER		
Totipotent cells in gonads or in embryonal rests	Mature teratoma	Immature teratoma

nervous system). On the other hand, malignant tumours grow rapidly, may ulcerate on the surface, invade locally into deeper tissues, may spread to distant sites (metastasis), and also produce systemic features such as weight loss, anorexia and anemia. In fact, two of the cardinal clinical features of malignant tumours are: *invasiveness and metastasis*.

The gross appearance of benign and malignant tumours may be quite variable and the features may not be diagnostic on the basis of gross appearance alone. However, certain distinctive features characterise almost all tumours—they have a different colour, texture and

consistency as compared to the surrounding tissue of origin. Gross terms such as papillary, fungating, infiltrating, haemorrhagic, ulcerative and cystic are used to describe the macroscopic appearance of the tumours.

■ *Benign tumours* are generally spherical or ovoid in shape. They are encapsulated or well-circumscribed, freely movable, more often firm and uniform, unless secondary changes like haemorrhage or infarction supervene (Fig. 20.1,A, E).

■ *Malignant tumours*, on the other hand, are usually irregular in shape, poorly-circumscribed and extend into the adjacent tissues. Secondary changes like haemor-

TABLE 20.2: Contrasting Features of Benign and Malignant Tumours.

FEATURES	BENIGN	MALIGNANT
I. CLINICAL AND GROSS FEATURES		
1. <i>Boundaries</i>	Encapsulated or well-circumscribed	Poorly-circumscribed and irregular
2. <i>Surrounding tissue</i>	Often compressed	Usually invaded
3. <i>Size</i>	Usually small	Often larger
4. <i>Secondary changes</i>	Occur less often	Occur more often
II. MICROSCOPIC FEATURES		
1. <i>Pattern</i>	Usually resembles the tissue of origin closely	Often poor resemblance to tissue of origin
2. <i>Basal polarity</i>	Retained	Often lost
3. <i>Pleomorphism</i>	Usually not present	Often present
4. <i>Nucleo-cytoplasmic ratio</i>	Normal	Increased
5. <i>Anisonucleosis</i>	Absent	Generally present
6. <i>Hyperchromatism</i>	Absent	Often present
7. <i>Mitoses</i>	May be present but are always typical mitoses	Mitotic figures increased and are generally atypical and abnormal
8. <i>Tumour giant cells</i>	May be present but without nuclear atypia	Present with nuclear atypia
9. <i>Chromosomal abnormalities</i>	Infrequent	Invariably present
10. <i>Function</i>	Usually well maintained	May be retained, lost or become abnormal
III. GROWTH RATE	Usually slow	Usually rapid
IV. LOCAL INVASION	Often compresses the surrounding tissues without invading or infiltrating them	Usually infiltrates and invades the adjacent tissues
V. METASTASIS	Absent	Frequently present
VI. PROGNOSIS	Local complications	Death by local and metastatic complications

rhage, infarction and ulceration are seen more often. Sarcomas typically have fish-flesh like consistency while carcinomas are generally firm (Fig. 20.1,C, G).

III. MICROSCOPIC FEATURES

For recognising and classifying the tumours, the microscopic characteristics of tumour cells are of greatest importance. These features which are appreciated in histologic sections are as under:

1. microscopic pattern;
2. cytomorphology of neoplastic cells (differentiation and anaplasia);
3. tumour angiogenesis and stroma; and
4. inflammatory reaction.

1. Microscopic Pattern

The tumour cells may be arranged in a variety of patterns in different tumours as under:

■ The *epithelial tumours* generally consist of acini, sheets, columns or cords of epithelial tumour cells that may be arranged in solid or papillary pattern (Fig. 20.1,B, D).

■ The *mesenchymal tumours* have mesenchymal tumour cells arranged as interlacing bundles, fascicles or whorls, lying separated from each other usually by the intercellular matrix substance such as hyaline material in leiomyoma (Fig. 20.1,E), cartilaginous matrix in chondroma, osteoid in osteosarcoma, reticulin network in soft tissue sarcomas etc (Fig. 20.1,H).

■ Certain tumours have *mixed patterns* e.g. teratoma arising from totipotent cells, pleomorphic adenoma of salivary gland (mixed salivary tumour), fibroadenoma of the breast, carcinosarcoma of the uterus and various other combinations of tumour types.

■ *Haematopoietic tumours* such as leukaemias and lymphomas often have none or little stromal support.

■ Generally, most benign tumours and low grade malignant tumours *reduplicate* the normal structure of origin more closely so that there is little difficulty in identifying and classifying such tumours (Fig. 20.1,B, F). However, anaplastic tumours differ greatly from the arrangement in normal tissue of origin of the tumour and may occasionally pose problems in classifying the tumour.

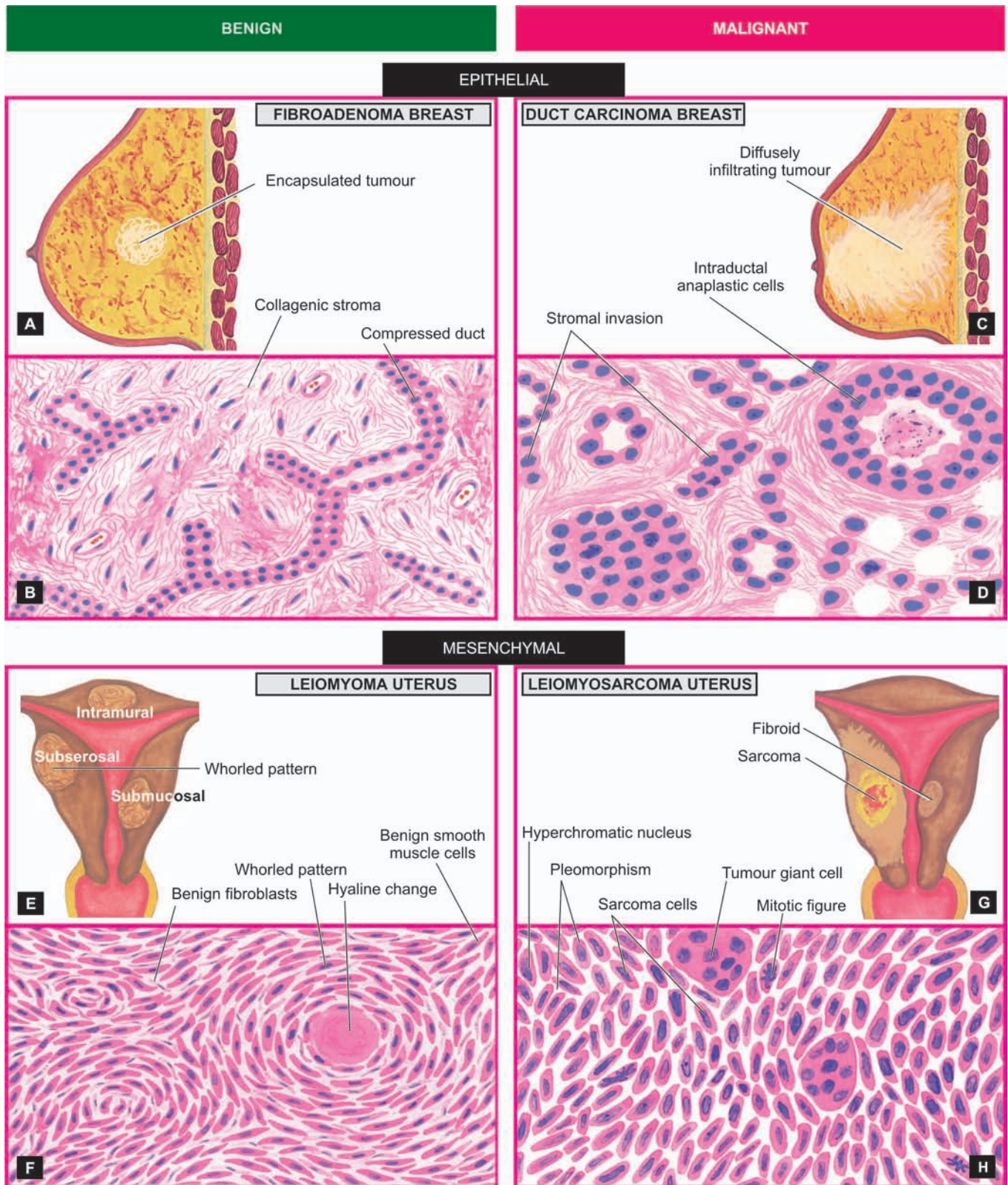


FIGURE 20.1

Gross and microscopic features of prototypes of benign (*left*) and malignant (*right*) tumours.

2. Cytomorphology of Neoplastic Cells (Differentiation and Anaplasia)

The neoplastic cell is characterised by morphologic and functional alterations, the most significant of which are 'differentiation' and 'anaplasia'.

■ **Differentiation** is defined as the extent of morphological and functional resemblance of parenchymal tumour cells to corresponding normal cells. If the deviation of neoplastic cell in structure and function is minimal as compared to normal cell, the tumour is described as '*well-differentiated*' such as most benign and low-grade malignant tumours. '*Poorly differentiated*', '*undifferentiated*' or '*dedifferentiated*' are synonymous terms for poor structural and functional resemblance to corresponding normal cell.

■ **Anaplasia** is lack of differentiation and is a characteristic feature of most malignant tumours. Depending upon the degree of differentiation, the extent of anaplasia is also variable i.e. poorly differentiated malignant tumours have high degree of anaplasia.

As a result of anaplasia, noticeable morphological and functional alterations in the neoplastic cells are observed. These are considered below and are diagrammatically illustrated in Fig. 20.2 (COLOUR PLATE VI: CL 24):

i) *Loss of polarity*. Normally, the nuclei of epithelial cells are oriented along the basement membrane which is termed as basal polarity. This property is based on cell adhesion molecules, particularly selectins. Early in malignancy, tumour cells lose their basal polarity so that the nuclei tend to lie away from the basement membrane.

ii) *Pleomorphism*. The term pleomorphism means variation in size and shape of the tumour cells. The extent of cellular pleomorphism generally correlates with the degree of anaplasia. Tumour cells are often bigger than normal but in some tumours they can be of normal size or smaller than normal.

iii) *N:C ratio*. Generally, the nuclei of malignant tumour cells show more conspicuous changes. Nuclei are enlarged disproportionate to the cell size so that the nucleocytoplasmic ratio is increased from normal 1:5 to 1:1.

iv) *Anisonucleosis*. Just like cellular pleomorphism, the nuclei too, show variation in size and shape in malignant tumour cells.

v) *Hyperchromatism*. Characteristically, the nuclear chromatin of malignant cell is increased and coarsely clumped. This is due to increase in the amount of

nucleoprotein resulting in dark-staining nuclei, referred to as hyperchromatism. Nuclear shape may vary, nuclear membrane may be irregular and nuclear chromatin is clumped along the nuclear membrane.

vi) *Nucleolar changes*. Malignant cells frequently have a prominent nucleolus or nucleoli in the nucleus reflecting increased nucleoprotein synthesis. This may be demonstrated as Nucleolar Organiser Region (NOR) by silver (Ag) staining called AgNOR material.

vii) *Mitotic figures*. The parenchymal cells of poorly-differentiated tumours often show large number of mitoses as compared with benign tumours and well-differentiated malignant tumours. As stated above, these appear as either normal or abnormal mitotic figures.

■ *Normal mitotic figures* may be seen in some non-neoplastic proliferating cells (e.g. haematopoietic cells of the bone marrow, intestinal epithelium, hepatocytes etc), in certain benign tumours and some low grade malignant tumours; in sections they are seen as a dark band of dividing chromatin at two poles of the nuclear spindle.

■ *Abnormal or atypical mitotic figures* are more important in malignant tumours and are identified as tripolar, quadripolar and multipolar spindles in malignant tumour cells.

viii) *Tumour giant cells*. Multinucleate tumour giant cells or giant cells containing a single large and bizarre nucleus, possessing nuclear characters of the adjacent tumour cells, are another important feature of anaplasia in malignant tumours.

ix) *Functional (Cytoplasmic) changes*. Structural anaplasia in tumours is accompanied with functional anaplasia as appreciated from the cytoplasmic constituents of the tumour cells. The functional abnormality in neoplasms may be quantitative, qualitative, or both.

■ Generally, benign tumours and better-differentiated malignant tumours continue to function well qualitatively, though there may be *quantitative* abnormality in the product e.g. large or small amount of collagen produced by benign tumours of fibrous tissue, keratin formation in well-differentiated squamous cell carcinoma. In more anaplastic tumours, there is usually quantitative fall in the product made by the tumour cells e.g. absence of keratin in anaplastic squamous cell carcinoma.

■ There may be both *qualitative and quantitative* abnormality of the cellular function in some anaplastic tumours e.g. multiple myeloma producing abnormal immunoglobulin in large quantities.

■ Endocrine tumours may cause excessive hormone production leading to characteristic clinical syndromes.

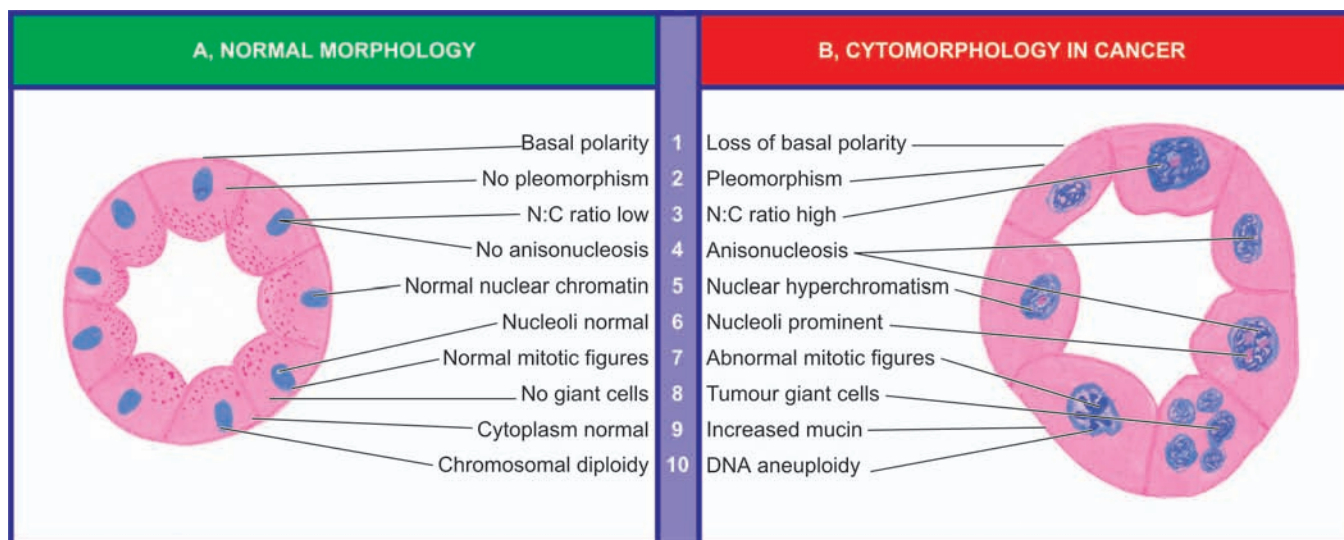


FIGURE 20.2

Diagrammatic representation of cytomorphologic features of neoplastic cells. Characteristics of cancer (B) in a gland are contrasted with the appearance of a normal mucous gland (A).

Besides the production of hormones by endocrine tumours, hormones or hormone-like substances may be produced by certain tumours quite unrelated to the endocrine glands. This property of tumours is called *ectopic hormone production* e.g. oat cell carcinoma of the lung can secrete ACTH and ADH; less often it may produce gonadotropin, thyrotropin, parathormone, calcitonin and growth hormone. Ectopic erythropoietin may be produced by carcinoma of kidneys, hepatocellular carcinoma and cerebellar haemangioblastoma.

x) Chromosomal abnormalities. All tumour cells have abnormal genetic composition and on division they transmit the genetic abnormality to their progeny. The chromosomal abnormalities are more marked in more malignant tumours which include deviations in both morphology and number of chromosomes. Most malignant tumours show *DNA aneuploidy*, often in the form of an increase in the number of chromosomes, reflected morphologically by the increase in the size of nuclei.

One of the most important examples of a consistent chromosomal abnormality in human malignancy is the presence of Philadelphia chromosome (named after the city in which it was first described) in 95% cases of chronic myeloid leukaemia. In this, part of the long arm of chromosome 9 is translocated to part of the long arm of chromosome 22 (t 9; 22). Other examples of neoplasms showing chromosomal abnormalities are Burkitt's lymphoma, acute lymphoid leukaemia, multiple myeloma, retinoblastoma, oat cell carcinoma, Wilms' tumour etc.

3. Tumour Angiogenesis and Stroma

The connective tissue alongwith its vascular network forms the supportive framework on which the parenchymal tumour cells grow and receive nourishment. In addition to variable amount of connective tissue and vascularity, the stroma may have nerves and metaplastic bone or cartilage but no lymphatics.

TUMOUR ANGIOGENESIS. In order to provide nourishment to growing tumour, new blood vessels are formed from pre-existing ones (angiogenesis). How this takes place is discussed later under molecular pathogenesis of cancer. However, related morphologic features are as under:

i) *Microvascular density.* The new capillaries add to the vascular density of the tumour which has been used as a marker to assess the rate of growth of tumours and hence grade the tumours. This is done by counting microvascular density in the section of the tumour.

ii) *Central necrosis.* However, if the tumour outgrows its blood supply as occurs in rapidly growing tumours or tumour angiogenesis fails, its core undergoes ischaemic necrosis.

TUMOUR STROMA. The collagenous tissue in the stroma may be scanty or excessive. In the former case, the tumour is soft and fleshy (e.g. in sarcomas, lymphomas), while in the latter case the tumour is hard and gritty (e.g. infiltrating duct carcinoma breast). Growth of fibrous tissue in tumour is stimulated by basic fibroblast growth factor (bFGF) elaborated by tumour cells.

- If the epithelial tumour is almost entirely composed of parenchymal cells, it is called *medullary* e.g. medullary carcinoma of the breast, medullary carcinoma of the thyroid.
- If there is excessive connective tissue stroma in the epithelial tumour, it is referred to as *desmoplasia* and the tumour is hard or *scirrhous* e.g. infiltrating duct carcinoma breast, linitis plastica of the stomach.

4. Inflammatory Reaction

At times, prominent inflammatory reaction is present in and around the tumours. It could be the result of ulceration in the cancer when there is secondary infection. The inflammatory reaction in such instances may be acute or chronic. However, some tumours show chronic inflammatory reaction, chiefly of lymphocytes, plasma cells and macrophages, and in some instances granulomatous reaction, in the absence of ulceration. This is due to cell-mediated immunologic response by the host in an attempt to destroy the tumour. In some cases, such an immune response improves the prognosis.

The *examples* of such reaction are: seminoma testis, malignant melanoma of the skin, lymphoepithelioma of the throat, medullary carcinoma of the breast, choriocarcinoma, Warthin's tumour of salivary glands etc.

IV. LOCAL INVASION (DIRECT SPREAD)

BENIGN TUMOURS. Most benign tumours form encapsulated or circumscribed masses that *expand and push aside* the surrounding normal tissues without actually invading, infiltrating or metastasising.

MALIGNANT TUMOURS. Malignant tumours also enlarge by expansion and some well-differentiated tumours may be partially encapsulated as well e.g. follicular carcinoma thyroid. But characteristically, they are distinguished from benign tumours by *invasion, infiltration and destruction* of the surrounding tissue, besides distant metastasis (described below). In general, tumours invade via the route of least resistance, though eventually most cancers recognise no anatomic boundaries. Often, cancers extend through tissue spaces, permeate lymphatics, blood vessels, perineural spaces and may penetrate a bone by growing through nutrient foramina. More commonly, the tumours invade thin-walled capillaries and veins than thick-walled arteries. Dense compact collagen, elastic tissue and cartilage are some of the tissues which are sufficiently resistant to invasion by tumours.

Mechanism of invasion of malignant tumours is discussed together with that of metastasis below.

V. METASTASIS (DISTANT SPREAD)

Metastasis (*meta* = transformation, *stasis* = residence) is defined as spread of tumour by invasion in such a way that discontinuous secondary tumour mass/masses are formed at the site of lodgement. *Metastasis and invasiveness are the two most important features to distinguish malignant from benign tumours.* Benign tumours do not metastasise while all the malignant tumours with a few exceptions like gliomas of the central nervous system and basal cell carcinoma of the skin, can metastasise. Generally, larger, more aggressive and rapidly-growing tumours are more likely to metastasise but there are numerous exceptions. About one third of malignant tumours at presentation have evident metastatic deposits while another 20% have occult metastasis.

Routes of Metastasis

Cancers may spread to distant sites by following pathways:

1. Lymphatic spread
2. Haematogenous spread
3. Other routes (Transcoelomic spread along epithelium-lined surfaces, spread via cerebrospinal fluid, implantation).

1. LYMPHATIC SPREAD. *In general, carcinomas metastasise by lymphatic route while sarcomas favour haematogenous route.* However, sarcomas may also spread by lymphatic pathway. The involvement of lymph nodes by malignant cells may be of two forms:

i) *Lymphatic permeation.* The walls of lymphatics are readily invaded by cancer cells and may form a continuous growth in the lymphatic channels called lymphatic permeation.

ii) *Lymphatic emboli.* Alternatively, the malignant cells may detach to form tumour emboli so as to be carried along the lymph to the next draining lymph node. The tumour emboli enter the lymph node at its convex surface and are lodged in the subcapsular sinus where they start growing (Fig. 20.3,C) (**COLOUR PLATE VIII: CL 31**). Later, of course, the whole lymph node may be replaced and enlarged by the metastatic tumour.

■ Generally, regional lymph nodes draining the tumour are invariably involved producing *regional nodal metastasis* e.g. from carcinoma breast to axillary lymph nodes, from carcinoma thyroid to lateral cervical lymph nodes, bronchogenic carcinoma to hilar and paratracheal lymph nodes etc (Fig. 20.3,A, B).

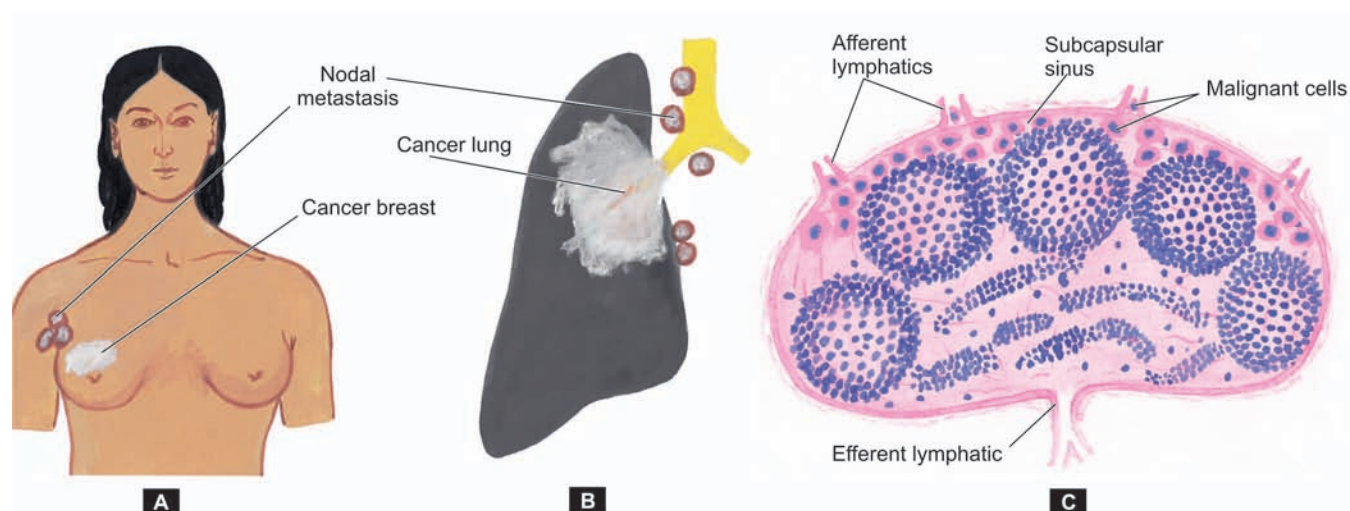


FIGURE 20.3

Regional nodal metastasis. A, Axillary nodes involved by carcinoma breast. B, Hilar and para-tracheal lymph nodes involved by bronchogenic carcinoma. C, Lymphatic spread begins by lodgement of tumour cells in subcapsular sinus via afferent lymphatics entering at the convex surface of the lymph node.

■ However, all regional nodal enlargements are not due to nodal metastasis because necrotic products of tumour and antigens may also incite regional lymphadenitis of *sinus histiocytosis*.

■ Sometimes lymphatic metastases do not develop first in the lymph node nearest to the tumour because of venous-lymphatic anastomoses or due to obliteration of lymphatics by inflammation or radiation, so called *skip metastasis*.

■ Other times, due to obstruction of the lymphatics by tumour cells, the lymph flow is disturbed and tumour cells spread against the flow of lymph causing *retrograde metastases* at unusual sites e.g. metastasis of carcinoma prostate to the supraclavicular lymph nodes, metastatic deposits from bronchogenic carcinoma to the axillary lymph nodes.

■ *Virchow's lymph node* is nodal metastasis preferentially to supraclavicular lymph node from cancers of abdominal organs e.g. cancer stomach, colon, and gall bladder.

It is believed that lymph nodes in the vicinity of tumour perform multiple roles—as initial barrier filter, and in destruction of tumour cells, while later provide fertile soil for growth of tumour cells.

Mechanism of lymphatic route of metastasis is discussed later under biology of invasion and metastasis.

2. HAEMATogenous SPREAD. *Blood-borne metastasis is the common route for sarcomas but certain carci-*

nomas also frequently metastasise by this mode, especially those of the lung, breast, thyroid, kidney, liver, prostate and ovary. The common sites for blood-borne metastasis are: the liver, lungs, brain, bones, kidney and adrenals, all of which provide 'good soil' for the growth of 'good seeds' (*seed-soil theory*). However, a few organs such as spleen, heart, skeletal muscle do not allow tumour metastasis to grow. Spleen is unfavourable site due to open sinusoidal pattern which does not permit tumour cells to stay there long enough to produce metastasis. In general, only a proportion of cancer cells are capable of clonal proliferation in the proper environment; others die without establishing a metastasis.

■ *Systemic veins* drain blood into vena cavae from limbs, head and neck and pelvis. Therefore, cancers of these sites more often metastasise to the lungs (**COLOUR PLATE VIII: CL 32**).

■ *Portal veins* drain blood from the bowel, spleen and pancreas into the liver. Thus, tumours of these organs frequently have secondaries in the liver.

■ *Arterial spread* of tumours is less likely because they are thick-walled and contain elastic tissue which is resistant to invasion. Nevertheless, arterial spread may occur when tumour cells pass through pulmonary capillary bed or through pulmonary arterial branches which have thin walls. Cancer of the lung may metastasise by pulmonary arterial route to kidneys, adrenals, bones, brain etc.

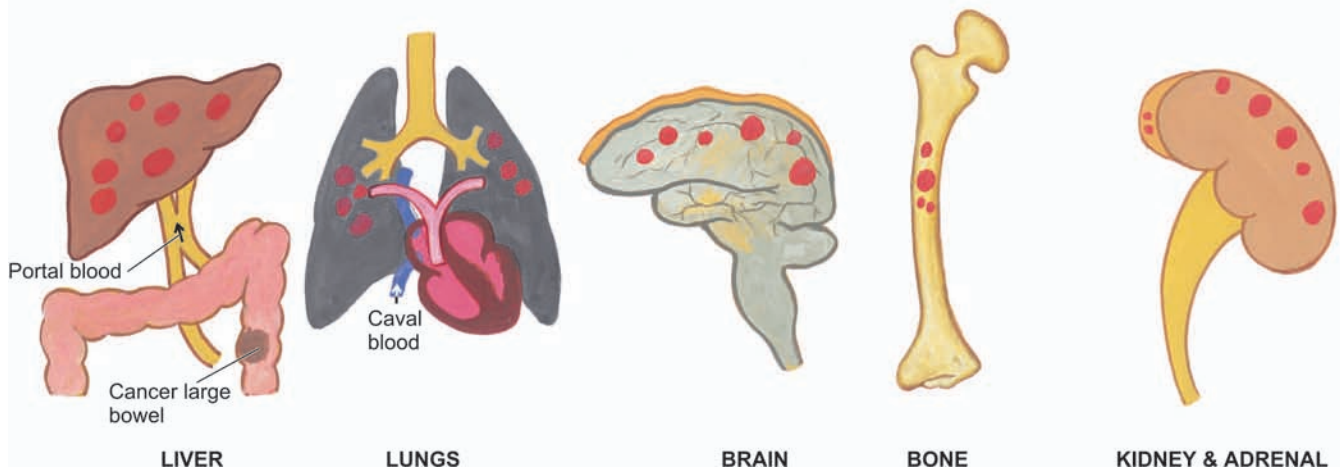


FIGURE 20.4

Gross appearance of haematogenous metastases at common sites.

■ *Retrograde spread* by blood route may occur at unusual sites due to retrograde spread after venous obstruction, just as with lymphatic metastases. Important examples are vertebral metastases in cancers of the thyroid and prostate.

Macroscopically, blood-borne metastases in an organ appear as multiple, rounded nodules of varying size, scattered throughout the organ (Fig. 20.4). Sometimes, the metastasis may grow bigger than the primary tumour. At times, metastatic deposits may come to attention first without an evident primary tumour. In such cases search for primary tumour may be rewarding, but rarely the primary tumour may remain undetected or occult. Metastatic deposits just like primary tumour may cause further dissemination via lymphatics and blood vessels.

Microscopically, the secondary deposits generally reproduce the structure of primary tumour. However, the same primary tumour on metastasis at different sites may show varying grades of differentiation, apparently due to the influence of local environment surrounding the tumour for its growth.

3. SPREAD ALONG BODY CAVITIES AND NATURAL PASSAGES. Uncommonly, some cancers may spread by *seeding* at other surfaces. These routes of distant spread are as under:

i) **Transcoelomic spread.** Certain cancers invade through the serosal wall of the coelomic cavity so that tumour fragments or clusters of tumour cells break off to be carried in the coelomic fluid and are implanted elsewhere in the body cavity. Peritoneal cavity is involved

most often, but occasionally pleural and pericardial cavities are also affected. Examples of transcoelomic spread are:

- a) *carcinoma of the stomach* seeding to both ovaries (Krukenberg tumour);
- b) *carcinoma of the ovary* spreading to the entire peritoneal cavity without infiltrating the underlying organs;
- c) *pseudomyxoma peritonei* is the gelatinous coating of the peritoneum from mucin-secreting carcinoma of the ovary or appendix; and
- d) *carcinoma of the bronchus and breast* seeding to the pleura and pericardium.

ii) **Spread along epithelium-lined surfaces.** It is unusual for a malignant tumour to spread along the epithelium-lined surfaces because intact epithelium and mucus coat are quite resistant to penetration by tumour cells. However, exceptionally a malignant tumour may spread through:

- a) the fallopian tube from the endometrium to the ovaries or *vice-versa*;
- b) through the bronchus into alveoli; and
- c) through the ureters from the kidneys into lower urinary tract.

iii) **Spread via cerebrospinal fluid.** Malignant tumour of the ependyma and leptomeninges may spread by release of tumour fragments and tumour cells into the CSF and produce metastases at other sites in the central nervous system.

iv) **Implantation.** Rarely, a tumour may spread by implantation by surgeon's scalpel, needles, sutures, or

may be implanted by direct contact such as transfer of cancer of the lower lip to the apposing upper lip.

MECHANISM AND BIOLOGY OF INVASION AND METASTASIS

The process of local invasion and distant spread (by lymphatic and haematogenous routes) discussed above involves passage through barriers before gaining access to the vascular lumen. This includes making the passage by the cancer cells by dissolution of extracellular matrix (ECM) at three levels— at the basement membrane of tumour itself, at the level of interstitial connective tissue, and at the basement membrane of microvasculature. The following steps are involved at the cell molecular level which are schematically illustrated in Fig. 20.5.

1. Aggressive clonal proliferation and angiogenesis.

The first step in the spread of cancer cells is the development of rapidly proliferating clone of cancer cells. This is explained on the basis of *tumour heterogeneity*, i.e. in the population of monoclonal tumour cells, a subpopulation or clone of tumour cells has the right biologic characteristics to complete the steps involved in the development of metastasis. Tumour angiogenesis plays a very significant role in metastasis since the new vessels formed as part of growing tumour are more vulnerable to invasion because these evolving vessels are directly in contact with cancer cells.

2. Tumour cell loosening. Normal cells remain glued to each other due to presence of cell adhesion molecules (CAMs), E (epithelial)-cadherin. In epithelial cancers there is either loss or inactivation of E-cadherin and also other CAMs of immunoglobulin superfamily, all of which results in loosening of cancer cells.

3. Tumour cell-ECM interaction. Loosened cancer cells now are attached to ECM proteins, mainly *laminin* and *fibronectin*. This attachment is facilitated due to profoundness of receptors on the cancer cells for both these proteins. There is also loss of *integrins*, the transmembrane receptors, further favouring invasion.

4. Degradation of ECM. Tumour cells overexpress *proteases* and matrix-degrading enzymes, *metalloproteinases* that includes collagenases and gelatinase, while the inhibitors of metalloproteinases are decreased. Another protease, cathepsin D, is also increased in certain cancers. These enzymes bring about dissolution of ECM—firstly basement membrane of tumour itself, then make way for tumour cells through the interstitial matrix, and finally dissolve the basement membrane of the vessel wall.

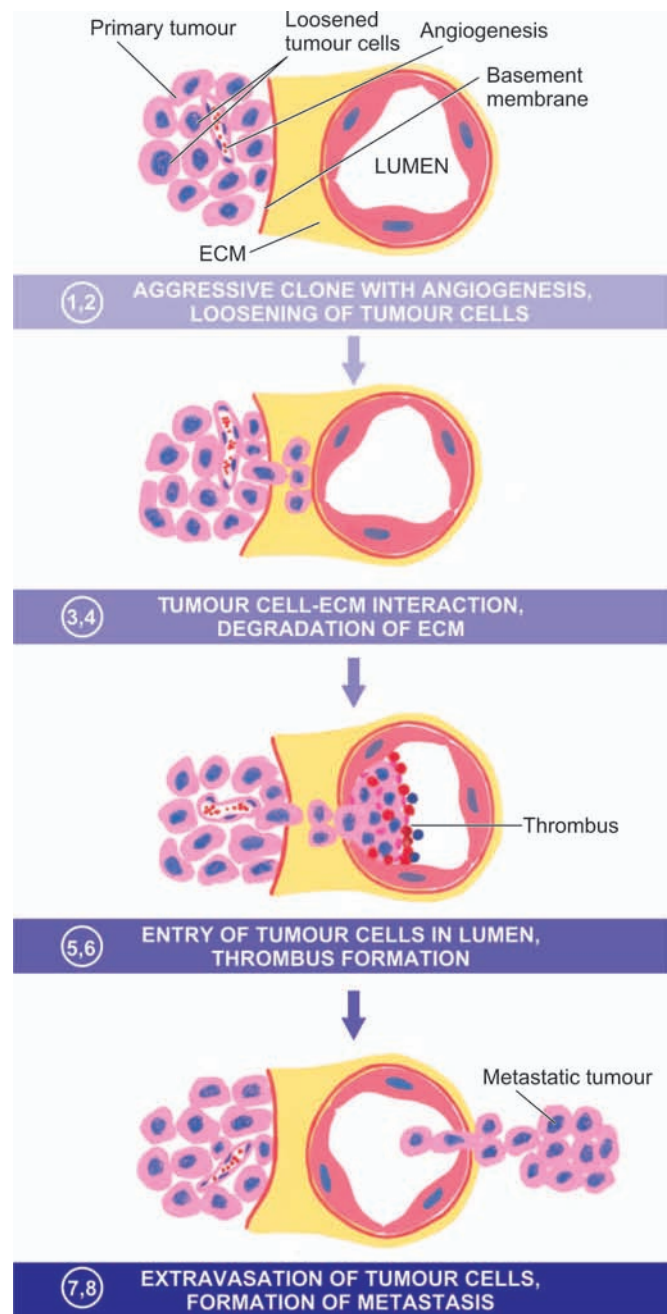


FIGURE 20.5

Mechanism and biology of local invasion and metastasis. The serial numbers in the figure correspond to their description in the text.

5. Entry of tumour cells into capillary lumen. The tumour cells after degrading the basement membrane are ready to migrate into lumen of capillaries or venules for which the following mechanisms play a role:

i) *Autocrine motility factor (AMF)* is a cytokine derived from tumour cells and stimulates receptor-mediated motility of tumour cells.

ii) *Cleavage products of matrix components* which are formed following degradation of ECM have properties of tumour cell chemotaxis, growth promotion and angiogenesis in the cancer.

After the malignant cells have migrated through the breached basement membrane, these cells enter the lumen of lymphatic and capillary channels.

6. Thrombus formation. The tumour cells protruding in the lumen of the capillary are now covered with constituents of the circulating blood and form the thrombus. Thrombus provides nourishment to the tumour cells and also protects them from the immune attack by the circulating host cells. In fact, normally a large number of tumour cells are released into circulation but they are attacked by the host immune cells. Actually a very small proportion of malignant cells (less than 0.1%) in the blood stream survive to develop into metastasis.

7. Extravasation of tumour cells. Tumour cells in the circulation (capillaries, venules, lymphatics) may mechanically block these vascular channels and attach to vascular endothelium. In this way, the sequence similar to local invasion is repeated and the basement membrane is exposed.

8. Survival and growth of metastatic deposit. The extravasated malignant cells on lodgement in the right environment grow further under the influence of growth factors produced by host tissues, tumour cells and by cleavage products of matrix components. These growth factors in particular include: PDGF, FGF, TGF- β and VEGF. The metastatic deposits grow further if the host immune defense mechanism fails to eliminate it. Metastatic deposits may further metastasise to the same organ or to other sites by forming emboli.

GRADING AND STAGING OF CANCER

'Grading' and 'staging' are the two systems to determine the prognosis and choice of treatment after a malignant tumour is detected. *Grading is defined as the macroscopic and microscopic degree of differentiation of the tumour, while staging means extent of spread of the tumour within the patient.*

Grading

Cancers may be graded grossly and microscopically. Gross features like exophytic or fungating appearance are indicative of less malignant growth than diffusely infiltrating tumours. However, grading is largely based on 2 important histologic features: *the degree of anaplasia,*

and the rate of growth. Based on these features, cancers are categorised from grade I as the most differentiated, to grade III or IV as the most undifferentiated or anaplastic. Many systems of grading have been proposed but the one described by *Broders* for dividing squamous cell carcinoma into 4 grades depending upon the degree of differentiation is followed for other malignant tumours as well. *Broders' grading* is as under:

Grade I: Well-differentiated (less than 25% anaplastic cells).

Grade II: Moderately-differentiated (25-50% anaplastic cells).

Grade III: Moderately-differentiated (50-75% anaplastic cells).

Grade IV: Poorly-differentiated or anaplastic (more than 75% anaplastic cells).

However, grading of tumours has several shortcomings. It is subjective and the degree of differentiation may vary from one area of tumour to the other. Therefore, it is common practice with pathologists to grade cancers in descriptive terms (e.g. well-differentiated, undifferentiated, keratinising, non-keratinising etc) rather than giving the tumours grade numbers.

Staging

The extent of spread of cancers can be assessed by 3 ways—by clinical examination, by investigations, and by pathologic examination of the tissue removed. Two important staging systems currently followed are: TNM staging and AJC staging.

TNM staging. (T for primary tumour, N for regional nodal involvement, and M for distant metastases) was developed by the UICC (Union Internationale Contre le Cancer, Geneva). For each of the 3 components namely T, N and M, numbers are added to indicate the extent of involvement, as under:

T_0 to T_4 : *In situ* lesion to largest and most extensive primary tumour.

N_0 to N_3 : No nodal involvement to widespread lymph node involvement.

M_0 to M_2 : No metastasis to disseminated haematogenous metastases.

AJC staging. (American Joint Committee staging) divides all cancers into stage 0 to IV, and takes into account all the 3 components of the preceding system (primary tumour, nodal involvement and distant metastases) in each stage.

TNM and AJC staging systems can be applied for staging most malignant tumours.



21

Etiology and Pathogenesis of Neoplasia

CANCER INCIDENCE

The overall incidence of cancer in a population or country is known by registration of all cancer cases (cancer registry) and by rate of death from cancer. Worldwide, it is estimated that about 20% of all deaths are cancer-related. There have been changing patterns in incidence of cancers in both the sexes and in different geographic locations as outlined below. Table 21.1 shows worldwide incidence (in descending order) of different forms of cancer in men, women, and children. As evident from the Table, some types of cancers are commoner in India while others are more common in the Western populations since etiologic factors are different.

EPIDEMIOLOGICAL FACTORS

A lot of clinical and experimental research and epidemiological studies have been carried out in the field of oncology so as to know the possible causes of cancer and mechanisms involved in transformation of a normal cell into a neoplastic cell. It is widely known that no single factor is responsible for development of tumours. The role of some factors in production of neoplasia is established while that of others is epidemiological and many others are still unknown.

Besides the etiologic role of some agents discussed later, the pattern and incidence of cancer depends upon the following:

- A) A large number of *predisposing epidemiologic factors or cofactors* which include a number of endogenous host factors and exogenous environmental factors.
- B) *Chronic non-neoplastic (pre-malignant) conditions*.
- C) *Role of hormones in cancer*.

A. Predisposing Factors

1. FAMILIAL AND GENETIC FACTORS. It has long been suspected that familial predisposition and heredity play a role in the development of cancers. In general, the risk of developing cancer in relatives of a known cancer patient is almost three times higher as compared to control subjects. The overall estimates suggest that genetic cancers comprise not greater than 5% of all cancers. Some of the common examples are as under:

i) Retinoblastoma. About 40% of retinoblastomas are familial and show an autosomal dominant inheritance. Carriers of such genetic composition have 10,000 times higher risk of developing retinoblastoma which is often bilateral. Such patients are predisposed to develop another primary malignant tumour, notably osteogenic sarcoma. Retinoblastoma susceptibility gene, *RB gene*, located on chromosome 13 was the first cancer-predisposing gene identified.

ii) Familial polyposis coli. This condition has autosomal dominant inheritance. The polypoid adenomas may be seen at birth or in early age. By the age of 50 years, almost 100% cases of familial polyposis coli develop cancer of the colon.

iii) Multiple endocrine neoplasia (MEN). A combination of adenomas of pituitary, parathyroid and pancreatic islets (MEN-I) or syndrome of medullary carcinoma thyroid, pheochromocytoma and parathyroid tumour (MEN-II) are encountered in families.

iv) Neurofibromatosis or von Recklinghausen's disease. This condition is characterised by multiple neurofibromas and pigmented skin spots (*cafe au lait spots*). These patients have family history consistent with autosomal dominant inheritance in 50% of patients.

TABLE 21.1: Most Common Malignant Tumours (In Descending Order).

MEN	WOMEN	CHILDREN
1. Lung (oral cavity in India)	Breast (cervix in India)	Acute leukaemias
2. Prostate	Lung	CNS tumours
3. Colon-rectum	Colon-rectum	Lymphomas
4. Leukaemia-lymphoma	Leukaemia-lymphoma	Neuroblastoma
5. Liver	Ovary	Bone sarcomas

v) **Cancer of the breast.** Female relatives of breast cancer patients have 2 to 3 times higher risk of developing breast cancer.

vi) **DNA-chromosomal instability syndromes.** These are a group of pre-neoplastic conditions having defect in DNA repair mechanism. A classical example is xeroderma pigmentosum, an autosomal recessive disorder, characterised by extreme sensitivity to ultraviolet radiation. The patients may develop various types of skin cancers such as basal cell carcinoma, squamous cell carcinoma and malignant melanoma.

2. RACIAL AND GEOGRAPHIC FACTORS. Differences in racial incidence of some cancers may be partly attributed to the role of genetic composition but are largely due to influence of the environment and geographic differences affecting the whole population such as climate, soil, water, diet, habits, customs etc. Some of the examples of racial and geographic variations in various cancers are as under:

i) **White Europeans and Americans** develop most commonly malignancies of the lung, breast, and colon. Liver cancer is uncommon in these races. Breast cancer is uncommon in Japanese women but is more common in American women.

ii) **Black Africans**, on the other hand, have more commonly cancers of the skin, penis, cervix and liver.

iii) **Japanese** have five times higher incidence of carcinoma of the stomach than the Americans.

iv) **South-East Asians**, especially of Chinese origin have more commonly nasopharyngeal cancer.

v) **Indians** of both sexes have higher incidence of carcinoma of the oral cavity and upper aerodigestive tract, while in females carcinoma of uterine cervix and of breast run parallel in incidence. Cancer of the liver in India is more due to viral hepatitis (HBV and HCV) and subsequent cirrhosis than due to alcoholism.

3. ENVIRONMENTAL AND CULTURAL FACTORS. Surprising as it may seem, we are surrounded by an environment of carcinogens which we eat, drink, inhale and touch. Some of the examples are given below:

i) **Cigarette smoking** is the single most important environmental factor implicated in the etiology of cancer of the oral cavity, pharynx, larynx, oesophagus, lungs, pancreas and urinary bladder.

ii) **Alcohol abuse** predisposes to the development of cancer of oropharynx, larynx, oesophagus and liver.

iii) **Alcohol and tobacco together** further accentuate the risk of developing cancer of the upper aerodigestive tract.

iv) **Cancer of the cervix** is linked to a number of factors such as age at first coitus, frequency of coitus, multiplicity of partners, parity etc. Sexual partners of circumcised males have lower incidence of cervical cancer than the partners of uncircumcised males.

v) **Penile cancer** is rare in the Jews and Muslims as they are customarily circumcised. Carcinogenic component of smegma appears to play a role in the etiology of penile cancer.

vi) **Betel nut cancer** of the cheek and tongue is quite common in some parts of India due to habitual practice of keeping the bolus of *paan* in a particular place in mouth for a long time.

vii) A large number of **industrial and environmental substances** are carcinogenic and are occupational hazard for some populations. These include exposure to substances like arsenic, asbestos, benzene, vinyl chloride, naphthylamine etc.

viii) **Certain constituents of diet** have also been implicated in the causation of cancer. Overweight individuals, deficiency of vitamin A and people consuming diet rich in animal fats and low in fibre content are more at risk of developing certain cancers such as colonic cancer. Diet rich in vitamin E, on the other hand, possibly has some protective influence by its antioxidant action.

4. AGE. Generally, cancers occur in older individuals past 5th decade of life, though there are variations in age incidence in different forms of cancers. It is not clear whether higher incidence of cancer in advanced age is due to alteration in the cells of the host, longer exposure to the effect of carcinogen, or decreased ability of the host immune response. Some tumours have two peaks of incidence e.g. acute leukaemias occur in children and in older age group. The biologic behaviour of tumours in children does not always correlate with histologic features. Besides acute leukaemias, *other tumours in infancy and childhood* are: neuroblastoma, nephroblastoma (Wilms' tumour), retinoblastoma, hepatoblastoma, rhabdomyosarcoma, Ewing's sarcoma, teratoma and CNS tumours.

5. SEX. Apart from the malignant tumours of organs peculiar to each sex, most tumours are generally more common in men than in women except cancer of the breast, gall bladder, thyroid and hypopharynx. Although there are geographic and racial variations, *cancer of the breast* is the commonest cancer in *women* throughout the world while *lung cancer* is the commonest cancer in *men*. The differences in incidence of certain cancers in the two sexes may be related to the presence of specific sex hormones.

B. Chronic Non-neoplastic (Pre-malignant) Conditions

Premalignant lesions are a group of conditions which predispose to the subsequent development of cancer. Such conditions are important to recognise so as to prevent the subsequent occurrence of an invasive cancer. Many of these conditions are characterised by morphologic changes in the cells such as increased nuclear-cytoplasmic ratio, pleomorphism of cells and nuclei, increased mitotic activity, poor differentiation, and sometimes accompanied by chronic inflammatory cells.

Some examples of premalignant lesions are given below:

1. Carcinoma *in situ* (intraepithelial neoplasia). When the cytological features of malignancy are present but the malignant cells are confined to epithelium without invasion across the basement membrane, it is called as carcinoma *in situ* or intraepithelial neoplasia (CIN). The common sites are:

- i) Uterine cervix at the junction of ecto and endocervix
- ii) Bowen's disease of the skin
- iii) Actinic or solar keratosis
- iv) Oral leukoplakia
- v) Intralobular and intraductal carcinoma of the breast.

The area involved in carcinoma *in situ* may be single and small, or multifocal. As regards the behaviour of CIN, it may regress and return to normal or may develop into invasive cancer. In some instances such as in cervical cancer, there is a sequential transformation from squamous metaplasia, to epithelial dysplasia, to carcinoma *in situ*, and eventually to invasive cancer.

2. Some benign tumours. Commonly, benign tumours do not become malignant. However, there are some exceptions e.g.

- i) Multiple villous adenomas of the large intestine have high incidence of developing adenocarcinoma.
- ii) Neurofibromatosis (von Recklinghausen's disease) may develop into sarcoma.

3. Miscellaneous conditions. Certain inflammatory and hyperplastic conditions are prone to development of cancer, e.g.

- i) Patients of long-standing ulcerative colitis are predisposed to develop colorectal cancer.
- ii) Cirrhosis of the liver has predisposition to develop hepatocellular carcinoma.
- iii) Chronic bronchitis in heavy cigarette smokers may develop cancer of the bronchus.
- iv) Chronic irritation from jagged tooth or ill-fitting denture may lead to cancer of the oral cavity.

- v) Squamous cell carcinoma developing in an old burn scar (Marjolin's ulcer).

C. Hormones and Cancer

Cancer is more likely to develop in organs and tissues which undergo proliferation under the influence of excessive hormonal stimulation. On cessation of hormonal stimulation, such tissues become atrophic. Hormone-sensitive tissues developing tumours are the breast, endometrium, myometrium, vagina, thyroid, liver, prostate and testis. Some examples of hormones influencing carcinogenesis in experimental animals and humans are given below:

1. OESTROGEN. Examples of oestrogen-induced cancers are as under:

i) In experimental animals. Induction of breast cancer in mice by administration of high-dose of oestrogen and reduction of the tumour development following oophorectomy is the most important example. Associated infection with mouse mammary tumour virus (MMTV, *Bittner milk factor*) has an added influence on the development of breast cancer in mice. Other cancers which can be experimentally induced in mice by oestrogens are squamous cell carcinoma of the cervix, connective tissue tumour of the myometrium, Leydig cell tumour of the testis in male mice, tumour of the kidney in hamsters, and benign as well as malignant tumours of the liver in rats.

ii) In humans. Women receiving oestrogen therapy and women with oestrogen-secreting granulosa cell tumour of the ovary have increased risk of developing endometrial carcinoma. Adenocarcinoma of the vagina is seen with increased frequency in adolescent daughters of mothers who had received oestrogen-therapy during pregnancy.

2. CONTRACEPTIVE HORMONES. The sequential types of oral contraceptives increase the risk of developing breast cancer. Other tumours showing a slightly increased frequency in women receiving contraceptive pills for long durations are benign tumours of the liver, and a few patients have been reported to have developed hepatocellular carcinoma.

3. ANABOLIC STEROIDS. Consumption of anabolic steroids by athletes to increase the muscle mass is not only unethical athletic practice but also increases the risk of developing benign and malignant tumours of the liver.

4. HORMONE-DEPENDENT TUMOURS. It has been shown in experimental animals that induction of hyperfunction of adenohypophysis is associated with increased risk of developing neoplasia of the target organs following preceding functional hyperplasia. There is tumour regression on removal of the stimulus for excessive hormonal secretion. A few examples of such phenomena are seen in humans:

- i) *Prostatic cancer* usually responds to the administration of oestrogens.
- ii) *Breast cancer* may regress with oophorectomy, hypophysectomy or on administration of male hormones.
- iii) *Thyroid cancer* may slow down in growth with administration of thyroxine that suppresses the secretion of TSH by the pituitary.

CARCINOGENESIS

Carcinogenesis or oncogenesis or tumorigenesis means mechanism of induction of tumours (*pathogenesis of cancer*); agents which can induce tumours are called *carcinogens (etiology of cancer)*. Since the time first ever carcinogen was identified, there has been ever-increasing list of agents implicated in etiology of cancer. There has been still greater accumulation in volumes of knowledge on pathogenesis of cancer, especially due to tremendous strides made in the field of molecular biology and genetics in the last decade.

The subject of etiology and pathogenesis of cancer is, therefore, discussed under the following 4 broad headings:

- A. Molecular pathogenesis of cancer (genes and cancer)
- B. Chemical carcinogens and chemical carcinogenesis
- C. Physical carcinogens and radiation carcinogenesis
- D. Biologic carcinogens and viral oncogenesis.

A. MOLECULAR PATHOGENESIS OF CANCER (GENES AND CANCER)

Basic Concept of Molecular Pathogenesis

The mechanism as to how a normal cell is transformed to a cancer cell is complex. At different times, attempts have been made to unravel this mystery by various mechanisms. In the last decade, there has been vast accumulation of literature to explain the pathogenesis of cancer at molecular level. The general concept of molecular mechanisms of cancer is briefly outlined below and diagrammatically shown in Fig. 21.1:

1. Monoclonality of tumours. There is strong evidence that most human cancers arise from a single clone of cells by genetic transformation or mutation. For example:

i) In a case of multiple myeloma (a malignant disorder of plasma cells), there is production of a single type of immunoglobulin or its chain as seen by monoclonal spike in serum electrophoresis.

ii) Due to inactivation of one of the two X-chromosomes in females (paternal or maternal derived), women are mosaics with two types of cell populations e.g. for glucose-6-phosphatase dehydrogenase (G6PD) isoenzyme A and B. It is observed that all the tumour cells in benign uterine tumours (leiomyoma) contain either A or B genotype of G6PD (i.e. the tumour cells are derived from a single progenitor clone of cell), while the normal myometrium is mosaic of both types of cells derived from A as well as B isoenzyme (Fig. 21.2).

2. Genetic theory of cancer. Cell growth of normal as well as abnormal types is under genetic control. In cancer, there is either abnormality in the genes of the

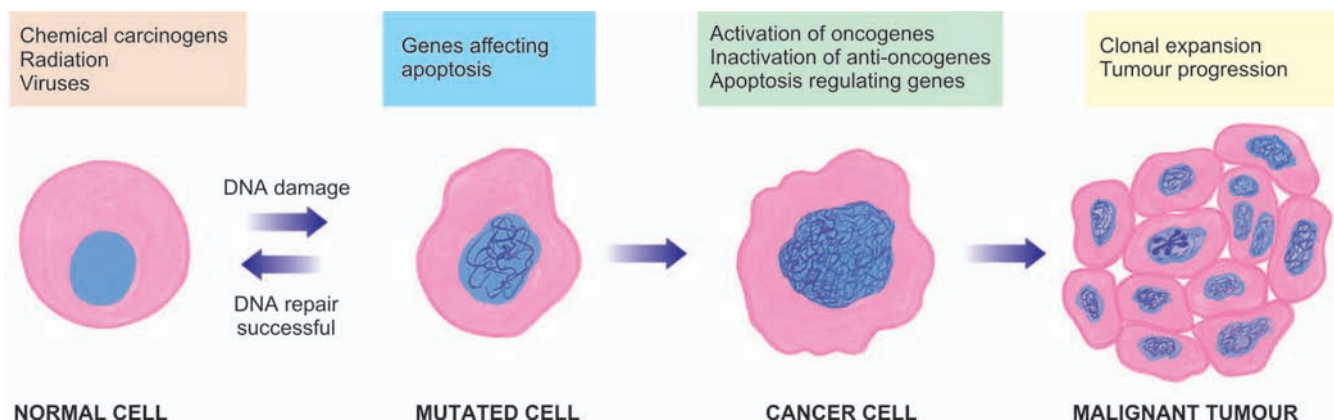


FIGURE 21.1

Schematic illustration to show molecular basis of cancer.

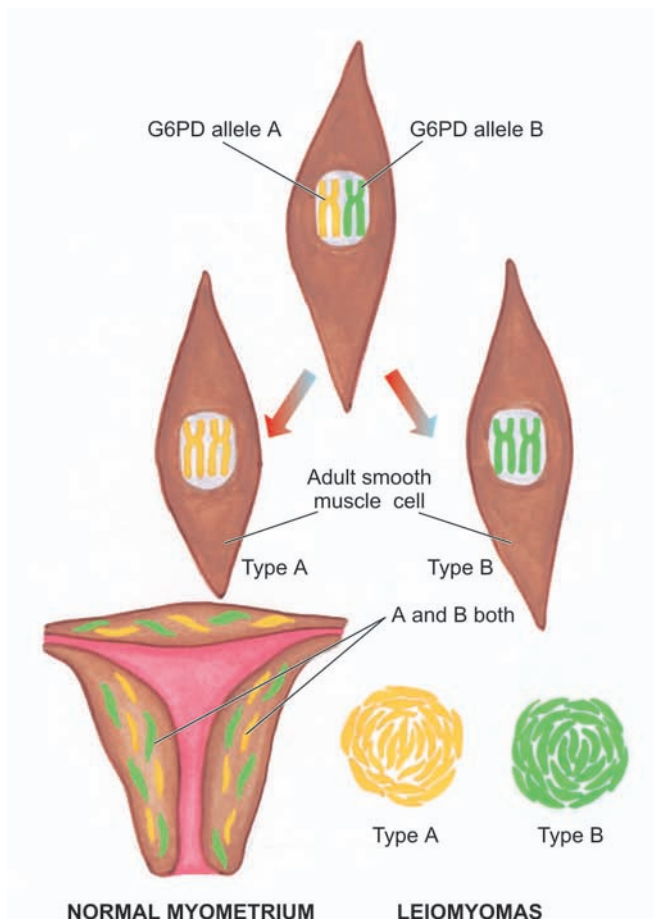


FIGURE 21.2

The monoclonal origin of tumour cells in uterine leiomyoma.

cell, or there are normal genes with abnormal expression. The abnormality in genetic composition may be from an inherited or induced mutation (induced by etiologic carcinogenic agents namely: chemicals, viruses, radiation). The mutated cells transmit their characters to the next progeny of cells and result in cancer.

3. Genetic regulators of normal and abnormal mitosis.

In normal cell growth, regulatory genes control mitosis as well as cell aging, terminating in cell death by apoptosis.

■ **In normal cell growth**, there are 4 regulatory genes:

- i) *Proto-oncogenes* are growth-promoting genes.
- ii) *Anti-oncogenes* are growth-inhibiting or growth suppressor genes.
- iii) *Apoptosis regulatory genes* control the programmed cell death.
- iv) *DNA repair genes* are those normal genes which regulate the repair of DNA damage that has occurred

during mitosis and also control the damage to proto-oncogenes and anti-oncogenes.

■ **In cancer**, the transformed cells are produced by abnormal cell growth due to genetic damage to these normal controlling genes. Thus, corresponding abnormalities are genetics, as under:

- i) *Activation of growth-promoting oncogenes* causing transformation of cell (mutant form of normal proto-oncogene in cancer is termed *oncogene*). Gene products of oncogenes are called *oncoproteins*. Oncogenes are considered *dominant* since they appear inspite of presence of normal proto-oncogenes.
- ii) *Inactivation of cancer-suppressor genes* (i.e. inactivation of anti-oncogenes) permitting the cellular proliferation of transformed cells. Anti-oncogenes are active in *recessive* form which mean that they are active only if both alleles are damaged.
- iii) *Abnormal apoptosis regulatory genes* which may act as oncogenes or anti-oncogenes. Accordingly, these genes may be active in dominant or recessive form.
- iv) *Failure of DNA repair genes* and thus inability to repair the DNA damage resulting in mutations.

4. Multi-step process of cancer growth and progression. Carcinogenesis is a gradual multi-step process involving many generations of cells. The various causes may act on the cell one after another (*multi-hit process*). The same process is also involved in further progression of the tumour. Ultimately, the cells so formed are genetically and phenotypically transformed cells having phenotypic features of malignancy—excessive growth, invasiveness and distant metastasis.

CANCER-RELATED GENES AND CELL GROWTH (HALLMARKS OF CANCER)

It is apparent from the above discussion that genes control the normal cellular growth, while in cancer these controlling genes are altered, typically by mutation. A large number of such cancer-associated genes have been described, each with a specific function in cell growth. Some of these genes are commonly associated in many tumours (e.g. *p53* or *TP53*), while others are specific to particular tumours. Therefore, it is considered appropriate to discuss the role of cancer-related genes with regard to their functions in cellular growth. Following are the *major genetic properties or hallmarks of cancer*:

1. Excessive and autonomous growth: Growth-promoting oncogenes.
2. Refractoriness to growth inhibition: Growth suppressing anti-oncogenes.

3. Escaping cell death by apoptosis: Genes regulating apoptosis and cancer.
4. Avoiding cellular aging: Telomeres and telomerase in cancer.
5. Continued perfusion of cancer: Cancer angiogenesis.
6. Invasion and distant metastasis: Cancer dissemination.
7. DNA damage and repair system: Mutator genes and cancer.
8. Cancer progression and tumour heterogeneity: Clonal aggressiveness.

These properties of cancer cells are described below in terms of molecular genetics and summed up in Fig. 21.3.

1. EXCESSIVE AND AUTONOMOUS GROWTH: GROWTH PROMOTING ONCOGENES

Mutated form of normal proto-oncogenes in cancer is called oncogenes. Oncogenes differ from 'normal genes' in following respects:

- mutation in the structure of gene;
- lacking the normal growth-promoting signals of proto-oncogenes; and

- they act by over-expression to promote autonomous and excessive cellular proliferation.

In general, activation of oncogenes in human tumours can occur by following mechanisms:

i) *Point mutations and deletion.* The most important example is *RAS* oncogene carried in many human tumours such as bladder cancer, pancreatic adenocarcinoma, cholangiocarcinoma.

ii) *Chromosomal translocation.* Mechanism of transfer of a portion of one chromosome to another is implicated in the pathogenesis of leukaemias and lymphomas e.g.

- Philadelphia chromosome seen in 95% cases of chronic myelogenous leukaemia in which *c-ABL* proto-oncogene on chromosome 9 is translocated to chromosome 22.

■ In 75% cases of Burkitt's lymphoma, translocation of *c-MYC* proto-oncogene from its site on chromosome 8 to a portion on chromosome 14.

iii) *Gene amplification.* Chromosomal alterations that result in increase in the number of copies of a gene is found in some examples of solid human tumours e.g.

- Neuroblastoma having *n-MYC* *HSR* region.
- *ERB-B* in breast and ovarian cancer.

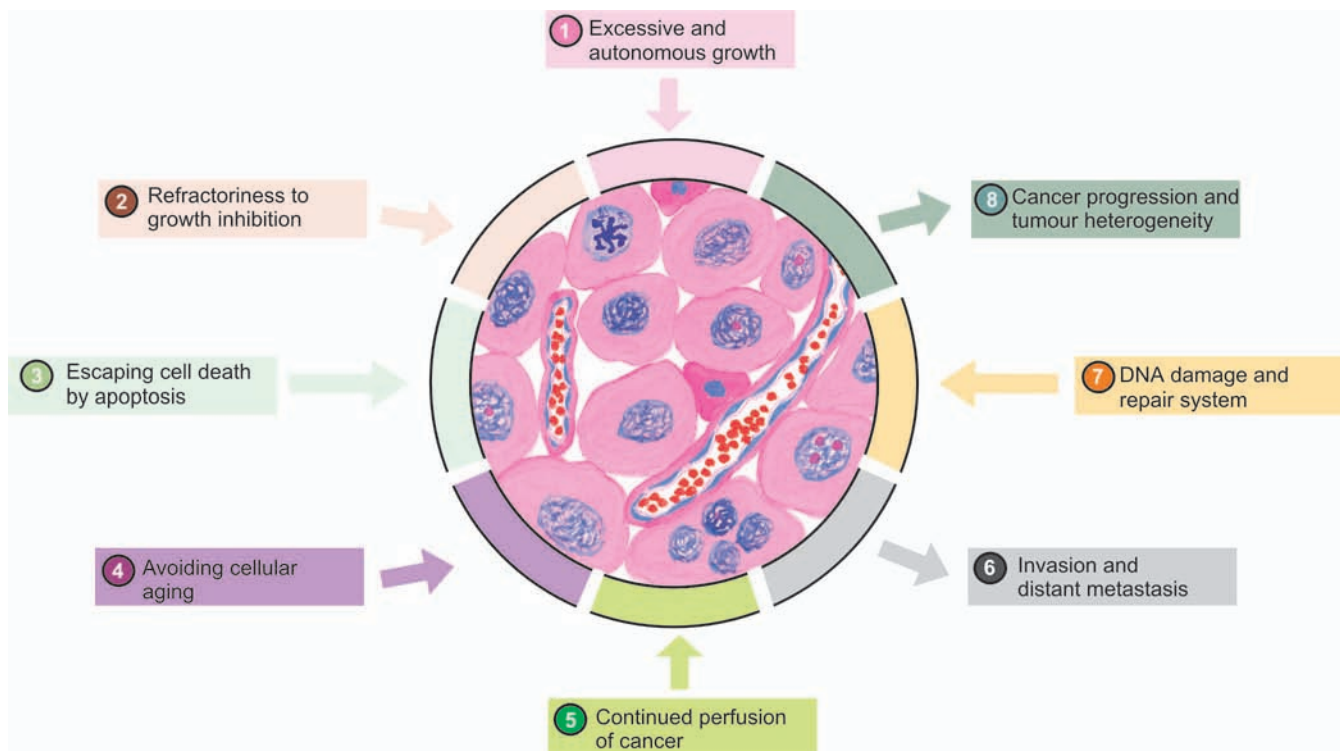


FIGURE 21.3

Schematic representation of major properties of cancer in terms of molecular carcinogenesis.

The steps in signal transduction for cell proliferation by oncogenes are discussed below in relation to mitosis in normal cell cycle:

i) Growth factors (GFs). These are polypeptides elaborated by many cells and normally act on another cell than the one which synthesised it to stimulate its proliferation i.e. *paracrine action*. However, a cancer cell may synthesise a GF and respond to it as well; this way cancer cells acquire growth self sufficiency. The examples of such GFs are as under:

- a) *Platelet-derived growth factor (PDGF)* in glioblastoma.
- b) *Transforming growth factor- α (TGF- α)* is over-expressed in sarcomas.
- c) *Fibroblast growth factor (FGF)* in cancer of the bowel and breast.

ii) Receptors for GFs. Many oncogenes encoding for GF receptors have been described which act more commonly by overexpression of normal GF than by mutation. For example:

- a) *ERB1* is an EGF receptor which acts by overexpression of normal GF receptor in squamous cell carcinoma.
- b) *HER2*, also called *ERB2*, is another EGF receptor which is overexpressed in breast cancer and carcinoma of lungs, ovary, stomach.

iii) Signal transduction proteins. The normal signal transduction proteins which transduce signal from the GF receptors on the cell surface to the nucleus of the cell is mutated in some cancers. The examples of such oncogenes are as under:

- a) *Mutated RAS gene.* This is the most common form of oncogene in human tumours. About a third of all human tumours carry mutated *RAS* gene (*RAS* for *Rat Sarcoma* gene where it was first described), seen in particular in carcinoma colon, lung and pancreas. Normally, the inactive form of *RAS* protein is GDP (guanosine diphosphate)-bound while the activated form is bound to guanosine triphosphate (GTP). GDP/GTP are homologous to G proteins and take part in signal transduction in a similar way just as G proteins act as 'on-off switch' for signal transduction. Normally, active *RAS* protein is inactivated by GTPase activity, while mutated *RAS* gene remains unaffected by GTPase and therefore, continues to signal the cell proliferation. Common mode of activation of *RAS* gene is by point mutation.
- b) *BCR-ABL hybrid gene.* *ABL* gene is a non-GF receptor proto-oncogene having tyrosine kinase activity. *ABL* gene from its normal location on chromosome 9 is translocated to chromosome 22 where it fuses with *BCR* (breakpoint cluster region) gene and forms an *ABL-BCR* hybrid gene which is more potent in signal transduction

pathway. *ABL-BCR* hybrid gene is seen in chronic myeloid leukaemia and some acute leukaemias.

iv) Nuclear regulatory molecules. The signal transduction pathway that started with GFs ultimately reaches the nucleus where it regulates DNA transcription. Out of various nuclear regulatory transcription proteins described, the most important is *MYC* gene, seen most commonly in human tumours. Normally *MYC* protein binds to the DNA and regulates the cell cycle by transcriptional activation and its levels fall immediately after cell enters the cell cycle. *MYC* oncogene, on the other hand, is associated with persistent or overexpression of *MYC* oncoproteins which, in turn, causes autonomous cell proliferation. The examples of tumours carrying *MYC* oncogene are:

- a) Burkitt's lymphoma in which mutation in *MYC* gene is due to *translocation* t(8;14).
- b) In cancer of lung, breast and colon, *MYC* gene is mutated due to *amplification*.

v) Cell cycle regulatory proteins. As discussed in Chapter 3, normally the cell cycle is under regulatory control of cyclins and cyclin-dependent kinases (CDKs) A, B, E and D. Cyclins are so named since they are cyclically synthesised during different phases of the cell cycle and their degradation is also cyclic. Cyclins activate as well as work together with CDKs, while many inhibitors of CDKs (CDKIs) are also known. Although all steps in the cell cycle are under regulatory controls, G1 $\rightarrow$ S phase is the most important checkpoint for regulation by oncogenes as well as anti-oncogenes (discussed below). Mutations in cyclins (in particular cyclin D) and CDKs (in particular CDK4) are most important growth promoting signals in cancers. The examples of tumours having such oncogenes are:

- a) *Overexpression of cyclin D* in cancers of breast, liver and mantle cell lymphoma.
- b) *Amplification of CDK4* in malignant melanoma, glioblastoma and sarcomas.

Various oncogenes in human tumours are listed in Table 21.2.

2. REFRACTORINESS TO GROWTH INHIBITION: GROWTH SUPPRESSING ANTI-ONCOGENES

The mutation of normal growth suppressor anti-oncogenes results in removal of the brakes for growth; thus the inhibitory effect to cell growth is removed and the abnormal growth continues unchecked. In other words, mutated anti-oncogenes behave like growth-promoting oncogenes.

As compared to the signals and signal transduction pathways for oncogenes described above, the steps in mechanisms of action by growth suppressors are not as

TABLE 21.2: Important Oncogenes, their Mechanism of Activation and Associated Human Tumours.

TYPE	ONCOGENE	ASSOCIATED HUMAN TUMOURS
1. Growth factors (GFs)	i) <i>PDGF</i> ii) <i>TGF-α</i> iii) <i>FGF</i>	Glioblastoma Sarcomas Ca bowel, breast
2. Receptors for GFs	i) <i>ERB B₁</i> ii) <i>HER₂ (α ERB₂)</i>	Squamous cell carcinoma Ca breast, ovary, stomach, lungs
3. Signal transduction proteins	<i>RAS</i> <i>BCR-ABL</i>	Common in 1/3rd human tumours, Ca lung, colon, pancreas CML, acute leukaemias
4. Nuclear regulatory molecules (Transcription proteins)	<i>MYC</i> (translocated) <i>MYC</i> (amplified)	Burkitt's lymphoma Ca lungs, breast, colon
5. Cell cycle regulatory proteins	<i>Cyclin D</i> <i>CDK4</i>	Ca breast, liver, mantle cell lymphoma Glioblastoma, melanoma, sarcomas

PDGF = platelet-derived growth factor; FGF = fibroblast growth factor; TGF- α = Transforming growth factor; CDK = cyclin-dependent kinase

well understood. In general, the point of action by anti-oncogenes is also G1 $\rightarrow$ S phase transition and probably act either by inducing the dividing cell from the cell cycle to enter into G0 (resting) phase, or by acting in a way that the cell lies in the post-mitotic pool losing its dividing capability.

Major anti-oncogenes implicated in human cancers are as under (Table 21.3):

i) ***RB* gene.** *RB* gene is located on long arm (q) of chromosome 13. This is the first ever tumour suppressor gene identified and thus has been amply studied. Normally, *RB* gene product is a nuclear transcription protein which is virtually present in every human cell. It exists in both an *active* and an *inactive* form. Active

RB gene protein acts to inhibit the cell cycle at G1 $\rightarrow$ S phase. Stimulation of the cell by growth factors renders the *RB* gene protein inactive and thus permits the cell to cross G1 $\rightarrow$ S phase.

The mutant form of *RB* gene is involved in retinoblastoma, the most common intraocular tumour in young children. The tumour occurs in two forms: sporadic and inherited/familial. More than half the cases are sporadic affecting one eye; these cases have acquired simultaneous mutation in both the alleles in retinal cells after birth. In inherited cases, all somatic cells inherit one mutant *RB* gene from a carrier parent, while the other allele gets mutated later. The latter genetic explanation given by Knudson forms the basis

TABLE 21.3: Tumour-suppressor Genes and Associated Human Tumours.

GENE	LOCATION	ASSOCIATED HUMAN TUMOURS
1. <i>RB</i>	Nucleus (q13)	Retinoblastoma, osteosarcoma
2. <i>TP53</i> (<i>p53</i>)	Nucleus (p17)	Most human cancers, common in ca. lung, head and neck, colon, breast
3. <i>TGF-β</i>	Extracellular GF	Ca pancreas, colon, stomach
4. <i>APC</i>	Cytosol	Ca colon
5. Others		
i) <i>WT 1 and 2</i>	Nucleus	Wilms' tumour
ii) <i>NF 1 and 2</i>	Plasma membrane	Neurofibromatosis type 1 and 2
iii) <i>BRCA 1 and 2</i>	Nucleus	Ca breast, ovary

of *two hit hypothesis* of inherited cancers. Besides retinoblastoma, children inheriting mutant *RB* gene have 200 times greater risk of development of other cancers in early adult life, most notably osteosarcoma; others are cancers of breast, colon and lungs.

ii) *TP53* gene (*p53*). Located on the short arm (p) of chromosome 17, *p53* gene (currently termed *TP53*) is normally a growth suppressor anti-oncogene.

The two major functions of *TP53* in the normal cell cycle are as under:

a) *In blocking mitotic activity:* *TP53* inhibits the cyclins and CDKs and prevents the cell to enter G1 phase transiently. This breathing time in the cell cycle is utilised by the cell to repair the DNA damage.

b) *In promoting apoptosis:* *TP53* acts together with another anti-oncogene, *RB* gene, and identifies the genes that have damaged DNA which cannot be repaired by inbuilt system. *TP53* directs such cells to apoptosis by activating apoptosis-inducing *BAX* gene, and thus bringing the defective cells to an end. This process operates in the cell cycle at G1 and G2 phases before the cell enters the S or M phase.

Because of these significant roles in cell cycle, *TP53* is called as 'protector of the genome'.

In its mutated form, *TP53* stops to act as growth suppressor and instead acts like an oncogene, *c-onc*. Majority of human cancers have either a mutation in *TP53* or its expression is up- or down-regulated. Some common examples of human cancers having defective *TP53* are: cancers of lung, head and neck, colon and breast. Besides, mutated *TP53* is also seen in the sequential development stages of cancer from hyperplasia to carcinoma *in situ* and into invasive carcinoma.

Like in *RB* gene, defect in one of the two alleles of *TP53* gene either by inheritance or by mutation, renders the individual prone to a second hit on the other normal allele and thus predisposes such an individual to cancers of multiple organs (breast, bone, brain, sarcomas etc), termed *Li-Fraumeni syndrome*.

iii) Transforming growth factor- β (*TGF- β*). Normally, *TGF- β* is significant inhibitor of cell proliferation, mainly by its action on G1 phase of cell cycle. Its mutant form impairs the growth inhibiting effect and thus permits cell proliferation. Examples of mutated form of *TGF- β* are seen in cancers of pancreas, colon, stomach etc.

iv) Adenomatous polyposis coli (*APC*) gene. The *APC* gene is normally inhibitory to mitosis, which is done by a cytoplasmic protein, β -catenin. β -catenin normally blocks the signal to the nucleus for activating mitosis. In colon cancer, *APC* gene is lost and thus the cancer cells continue to undergo mitosis without the inhibitory influence of β -catenin.

v) Other anti-oncogenes. A few other tumour-suppressor genes having mutated germline in various tumours are as under:

a) *Wilms' tumour (WT) gene:* *WT* gene normally prevents neoplastic proliferation of cells in embryonic kidney. Mutant form of *WT-1* and *2* are seen in hereditary Wilms' tumour.

b) *Neurofibroma (NF) gene:* *NF* genes normally prevent proliferation of Schwann cells. Two mutant forms are described: *NF1* and *NF2* seen in neurofibromatosis type 1 and type 2.

c) *BRCA 1 and BRCA 2 genes:* These are breast (*BR*) cancer (*CA*) susceptibility genes, especially in inherited cases of breast cancer.

3. ESCAPING CELL DEATH BY APOPTOSIS: GENES REGULATING APOPTOSIS AND CANCER

Besides the role of mutant forms of growth-promoting oncogenes and growth-suppressing anti-oncogenes, another mechanism of tumour growth is by escaping apoptosis. Apoptosis in normal cell is guided by cell death receptor, *CD95*, resulting in DNA damage. Besides, there is role of some other pro-apoptotic factors (*BAD*, *BAX*, *BID* and *TP53*) and apoptosis-inhibitors (*BCL2*, *BCL-X*).

In cancer cells, the function of apoptosis is interfered due to mutations in the above genes which regulate apoptosis in the normal cell. The examples of tumours by this mechanism are as under:

a) ***BCL2* gene** is seen in normal lymphocytes, but its mutant form with characteristic translocation was first described in B-cell lymphoma and hence the name. It is also seen in many other human cancers such as that of breast, thyroid and prostate. Mutation in *BCL2* gene removes the apoptosis-inhibitory control on cancer cells, thus more live cells undergoing mitosis contributing to tumour growth.

b) ***CD95* receptors** are depleted in hepatocellular carcinoma and hence the tumour cells escape apoptosis.

4. AVOIDING CELLULAR AGING: TELOMERES AND TELOMERASE IN CANCER

As discussed in pathology of aging in Chapter 3, after each mitosis (cell doubling) there is progressive shortening of telomeres which are the terminal tips of chromosomes. Telomerase is the RNA enzyme that helps in repair of such damage to DNA and maintains normal telomere length in successive cell divisions. However, it has been seen that after repetitive mitosis for a maximum of 60 to 70 times, telomeres are lost in normal cells and the cells cease to undergo mitosis. Telomerase

is active in normal stem cells but not in normal somatic cells.

Cancer cells in most malignancies have markedly upregulated telomerase enzyme, and hence telomere length is maintained. Thus, cancer cells avoid aging, mitosis does not slow down or cease, thereby immortalising the cancer cells.

5. CONTINUED PERFUSION OF CANCER: TUMOUR ANGIOGENESIS

Cancers can only survive and thrive if the cancer cells are adequately nourished and perfused, as otherwise they cannot grow further. Neovascularisation in the cancers not only supplies the tumour with oxygen and nutrients, but the newly formed endothelial cells also elaborate a few growth factors for progression of primary as well as metastatic cancer. The stimulus for angiogenesis is provided by the release of various factors:

i) **Promoters of tumour angiogenesis** include the most important *vascular endothelial growth factor (VEGF)* (released from genes in the parenchymal tumour cells) and *basic fibroblast growth factor (bFGF)*.

ii) **Anti-angiogenesis factors** inhibiting angiogenesis include *thrombospondin-1* (also produced by tumour cells themselves), *angiostatin*, *endostatin* and *vasculostatin*. Mutated form of *TP53* gene in both alleles in various cancers results in removal of anti-angiogenic role of thrombospondin-1, thus favouring continued angiogenesis.

6. INVASION AND DISTANT METASTASIS: CANCER DISSEMINATION

One of the most important characteristic of cancers is invasiveness and metastasis. The mechanisms involved in the biology of invasion and metastasis are discussed already along with spread of tumours.

7. DNA DAMAGE AND REPAIR SYSTEM: MUTATOR GENES AND CANCER

Normal cells during complex mitosis suffer from minor damage to the DNA which is detected and repaired before mitosis is completed so that integrity of the genome is maintained. Similarly, small mutational damage to the dividing cell by exogenous factors (e.g. by radiation, chemical carcinogens etc) is also repaired. *TP53* gene is held responsible for detection and repair of DNA damage. However, if this system of DNA repair is defective as happens in some inherited mutations (mutator genes), the defect in DNA is passed to the next progeny of cells and cancer results.

The examples of mutator genes exist in the following inherited disorders associated with increased propensity to cancer:

i) *Hereditary non-polyposis colon cancer* (Lynch syndrome) is characterised by hereditary predisposition to develop colorectal cancer. It is due to defect in genes involved in DNA mismatch repair which results in accumulation of errors in the form of mutations in many genes.

ii) *Ataxia telangiectasia (AT)* has *ATM* (M for mutated) gene. These patients have multiple cancers besides other features such as cerebellar degeneration, immunologic derangements and oculo-cutaneous manifestations.

iii) *Xeroderma pigmentosum* is an inherited disorder in which there is defect in DNA repair mechanism. Upon exposure to sunlight, the UV radiation damage to DNA cannot be repaired. Thus, such patients are more prone to various forms of skin cancers.

iv) *Bloom syndrome* is an example of damage by ionising radiation which cannot be repaired due to inherited defect and the patients have increased risk to develop cancers, particularly leukaemia.

v) *Hereditary breast cancer* patients having mutated *BRCA1* and *BRCA2* genes carrying inherited defect in DNA repair mechanism. These patients are not only predisposed to develop breast cancer but also cancers of various other organs.

8. CANCER PROGRESSION AND HETEROGENEITY: CLONAL AGGRESSIVENESS

Another feature of note in biology of cancers is that with passage of time they become more aggressive; this property is termed *tumour progression*. Clinical parameters of cancer progression are: increasing size of the tumour, higher histologic grade (as seen by poorer differentiation and greater anaplasia), areas of tumour necrosis (i.e. tumour outgrows its blood supply), invasiveness and distant metastasis.

In terms of molecular biology, this attribute of cancer is due to the fact that with passage of time cancer cells acquire more and more *heterogeneity*. This means that though cancer cells remain monoclonal in origin, they acquire more and more mutations which in turn produce multiple-mutated subpopulations of more aggressive clones of cancer cells (i.e. heterogeneous cells) in the growth which have tendency to invade, metastasise and be refractory to hormonal influences. Some of these mutations in fact may kill the tumour cells as well.

The above properties of cancer cells are schematically illustrated in Fig. 21.4.

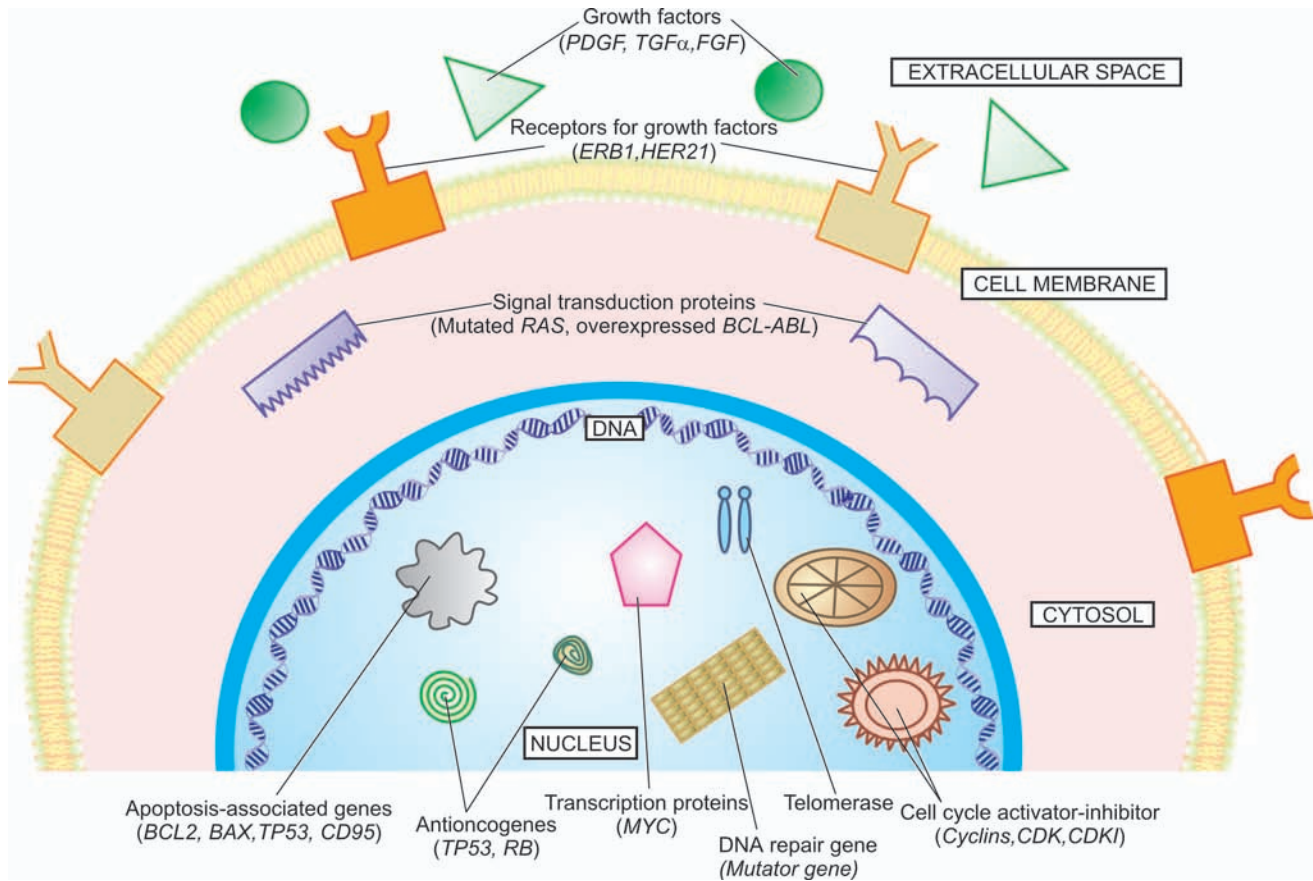


FIGURE 21.4

Schematic representation of activation-inactivation of cancer-associated genes in cell cycle.

B. CHEMICAL CARCINOGENESIS

The first ever evidence of any cause for neoplasia came from the observation of Sir Percival Pott in 1775 that there was higher incidence of cancer of the scrotum in chimney-sweeps in London than in the general population. This invoked wide interest in soot and coal tar as possible carcinogenic agent and the possibility of other occupational cancers. The first successful experimental induction of cancer was produced by two Japanese workers (Yamagiwa and Ichikawa) in 1914 in the rabbit's skin by repeatedly painting with coal tar. Since then the list of chemical carcinogens which can experimentally induce cancer in animals and have epidemiological evidence in causing human neoplasia, is ever increasing.

Stages in Chemical Carcinogenesis

The induction of cancer by chemical carcinogens occurs after a delay—weeks to months in the case of experi-

mental animals, and often several years in man. Other factors that influence the induction of cancer are the dose and mode of administration of carcinogenic chemical, individual susceptibility and various predisposing factors.

Basic mechanism of chemical carcinogenesis is by induction of mutation in the proto-oncogenes and anti-oncogenes. The phenomena of cellular transformation by chemical carcinogens (as also other carcinogens) is a progressive process involving 2 distinct sequential stages: initiation and promotion (Fig. 21.5).

1. INITIATION OF CARCINOGENESIS

Initiation is the first stage in carcinogenesis induced by initiator chemical carcinogens. The change can be produced by a single dose of the initiating agent for a short time, though larger dose for longer duration is more effective. The change so induced is sudden, irreversible and permanent. Chemical carcinogens acting

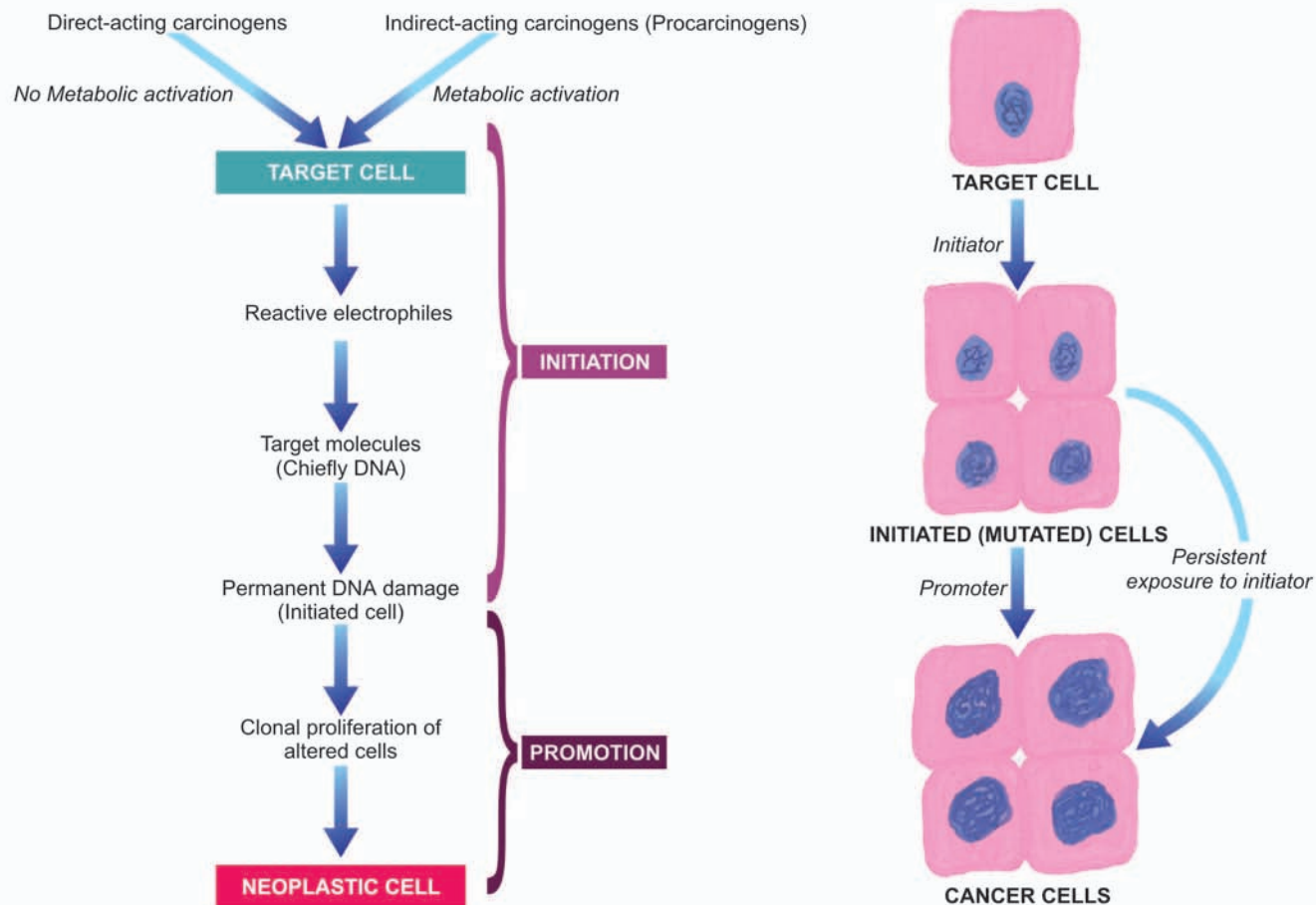


FIGURE 21.5

Sequential stages in chemical carcinogenesis (left) in evolution of cancer (right).

as initiators of carcinogenesis can be grouped into 2 categories (Table 21.4):

I. Direct-acting carcinogens. These are a few chemical substances (e.g. alkylating agents, acylating agents) which can induce cellular transformation without undergoing any prior metabolic activation.

II. Indirect-acting carcinogens or procarcinogens. These require metabolic conversion within the body so as to become 'ultimate' carcinogens having carcinogenicity e.g. polycyclic aromatic hydrocarbons, aromatic amines, azo dyes, naturally-occurring products and others.

In either case, the following steps are involved in transforming 'the target cell' into 'the initiated cell':

a) Metabolic activation. Vast majority of chemical carcinogens are indirect-acting or procarcinogens requiring metabolic activation, while direct-acting

carcinogens do not require this activation. The indirect-acting carcinogens are activated in the liver by the mono-oxygenases of the cytochrome P-450 system in the endoplasmic reticulum. In some circumstances, the procarcinogen may be detoxified and rendered inactive metabolically.

In fact, following 2 requirements determine the carcinogenic potency of a chemical:

- Balance between activation and inactivation reaction of the carcinogenic chemical.
- Genes that code for cytochrome P450-dependent enzymes involved in metabolic activation. For example, a genotype carrying susceptibility gene *CYP1A1* for the enzyme system has far higher incidence of lung cancer in light smokers as compared to those not having this permissive gene.

Besides these two, additional factors such as age, sex and nutritional status of the host also play some

TABLE 21.4: Important Chemical Carcinogens.

CARCINOGEN	TUMOUR
I. DIRECT-ACTING CARCINOGENS	
a) <i>Alkylating agents</i>	• Lymphomas • Leukaemias
• Anti-cancer drugs (e.g. cyclophosphamide, chlorambucil, busulfan, melphalan, nitrosourea etc) • β-propiolactone • Epoxides	
b) <i>Acylation agents</i>	
• Acetyl imidazole • Dimethyl carbamyl chloride	
II. INDIRECT-ACTING CARCINOGENS (PROCARCINOGENS):	
a) <i>Polycyclic, aromatic hydrocarbons</i> (in tobacco, smoke, fossil fuel, soot, tar, minerals oil, smoked animal foods, industrial and atmospheric pollutants)	• Lung cancer • Skin cancer • Cancer of oral cavity • Sarcoma
• Anthracenes (benza-, dibenza-, dimethyl benza-) • Benzapyrene • Methylcholanthrene	
b) <i>Aromatic amines and azo-dyes</i>	
• β-naphthylamine • Benzidine • Azo-dyes (e.g. butter yellow, scarlet red etc)	• Bladder cancer • Hepatocellular carcinoma
c) <i>Naturally-occurring products</i>	• Hepatocellular carcinoma
• Aflatoxin B1 • Actinomycin D • Mitomycin C • Safrole • Betel nuts	
d) <i>Miscellaneous</i>	
• Nitrosamines and nitrosamides • Vinyl chloride monomer • Asbestos • Arsenical compounds • Metals (e.g. nickel, lead, cobalt, chromium etc) • Insecticides, fungicides (e.g. aldrin, dieldrin, chlordane etc) • Saccharin and cyclomates	• Gastric carcinoma • Haemangiosarcoma of liver • Bronchogenic carcinoma, mesothelioma • Epidermal hyperplasia, basal cell carcinoma • Lung cancer • Cancer in experimental animals

role in determining response of the individual to chemical carcinogen.

b) Reactive electrophiles. While direct-acting carcinogens are intrinsically electrophilic, indirect-acting substances become electron-deficient after metabolic activation i.e. reactive electrophiles. Following this step, both types of chemical carcinogens behave alike and their reactive electrophiles bind to electron-rich portions of other molecules of the cell such as DNA, RNA and other proteins.

c) Target molecules. The primary target of electrophiles is DNA, producing mutagenesis. The change in DNA

may lead to 'the initiated cell' or some form of cellular enzymes may be able to repair the damage in DNA. The classic example of the latter situation as xeroderma pigmentosum, a precancerous condition, in which there is hereditary defect in DNA repair mechanism of the cell. The carcinogenic potential of a chemical can be tested *in vitro* by Ames' test for mutagenesis (described later).

Any gene may be the target molecule in the DNA for the chemical carcinogen. However, on the basis of chemically induced cancers in experimental animals and epidemiologic studies in human beings, it has been observed that most frequently affected oncogene is RAS

gene mutation and anti-oncogene (tumour suppressor) is *TP53* gene mutation.

d) The initiated cell. The unrepaired damage produced in the DNA of the cell becomes permanent and fixed only if the altered cell undergoes at least one cycle of proliferation. This results in transferring the change to the next progeny of cells so that the DNA damage becomes *permanent* and *irreversible*, which are the characteristics of the initiated cell, vulnerable to the action of promoters of carcinogenesis.

The stimulus for proliferation may come from regeneration of surviving cells, dietary factors, hormone-induced hyperplasia, viruses etc. A few examples are the occurrence of hepatocellular carcinoma in cases of viral hepatitis, association of endometrial hyperplasia with endometrial carcinoma, effect of oestrogen in breast cancer.

2. PROMOTION OF CARCINOGENESIS

Promotion is the next sequential stage in the chemical carcinogenesis. Promoters of carcinogenesis are substances such as phorbol esters, phenols, hormones, artificial sweeteners and drugs like phenobarbital. They differ from initiators in the following respects:

- i) They do not produce sudden change.
- ii) They require application or administration, as the case may be, *following* initiator exposure, for sufficient time and in sufficient dose.
- iii) The change induced may be reversible.
- iv) They do not damage the DNA *per se* and are thus not mutagenic but instead enhance the effect of direct-acting carcinogens or procarcinogens.
- v) Tumour promoters act by further clonal proliferation and expansion of initiated (mutated) cells, and have reduced requirement of growth factor especially after *RAS* gene mutation.

It may be mentioned here that persistent and sustained application/exposure of the cell to initiator alone unassociated with subsequent application of promoter may also result in cancer. But the *vice versa* does not hold true since neither application of promoter alone, nor its application prior to exposure to initiator carcinogen, would result in transformation of target cell.

Carcinogenic Chemicals in Humans

The list of diverse chemical compounds which can produce cancer in experimental animals is a long one but only some of them have sufficient epidemiological evidence in human neoplasia.

Depending upon the mode of action of carcinogenic chemicals, they are divided into 2 broad groups: initiators and promoters (Table 21.4).

1. INITIATOR CARCINOGENS

Chemical carcinogens which can initiate the process of neoplastic transformation are further categorised into 2 subgroups—direct-acting and indirect-acting carcinogens or procarcinogens.

I. DIRECT-ACTING CARCINOGENS. These chemical carcinogens do not require metabolic activation and fall into 2 classes:

a) Alkylating agents. This group includes mainly various anti-cancer drugs (e.g. cyclophosphamide, chlorambucil, busulfan, melphalan, nitrosourea etc), β -propiolactone and epoxides. They are weakly carcinogenic and are implicated in the etiology of the lymphomas and leukaemias in human beings.

b) Acylating agents. The examples are acetyl imidazole and dimethyl carbamyl chloride.

II. INDIRECT-ACTING CARCINOGENS (PRO-CARCINOGENS). These are chemical substances which require prior metabolic activation before becoming potent 'ultimate' carcinogens. This group includes vast majority of carcinogenic chemicals. It includes the following 4 categories:

a) Polycyclic aromatic hydrocarbons. They comprise the largest group of common procarcinogens which, after metabolic activation, can induce neoplasia in many tissues in experimental animals and are also implicated in a number of human neoplasms. They cause different effects by various modes of administration e.g. by topical application may induce skin cancer, by subcutaneous injection may cause sarcomas, inhalation produces lung cancer, when introduced in different organs by parenteral/metabolising routes may cause cancer of that organ.

Main sources of polycyclic aromatic hydrocarbons are: combustion and chewing of tobacco, smoke, fossil fuel (e.g. coal), soot, tar, mineral oil, smoked animal foods, industrial and atmospheric pollutants. Important chemical compounds included in this group are: anthracenes (benza-, dibenza-, dimethyl benza-), benza-pyrene and methylcholanthrene. The following examples have evidence to support the etiologic role of these substances:

■ **Smoking:** There is 20 times higher incidence of lung cancer in smokers of 2 packs (40 cigarettes) per day for 20 years.

■ **Skin cancer:** Direct contact of polycyclic aromatic hydrocarbon compounds with skin is associated with higher incidence of skin cancer. For example, the natives of Kashmir carry an earthen pot containing embers, the *kangri*, under their clothes close to abdomen for purposes of warmth, and skin cancer of the abdominal wall termed *kangri cancer* is common among them.

■ **Tobacco and betel nut chewing:** Cancer of the oral cavity is more common in people chewing tobacco and betel nuts. The *chutta* is a cigar that is smoked in South India (in Andhra Pradesh) with the lighted end in the mouth (i.e. reversed smoking) and such individuals have higher incidence of cancer of the mouth.

b) Aromatic amines and azo-dyes. This category includes the following substances implicated in chemical carcinogenesis:

■ **β -naphthylamine** in the causation of bladder cancer, especially in aniline dye and rubber industry workers.

■ **Benzidine** in the induction of bladder cancer.

■ **Azo-dyes** used for colouring foods (e.g. butter and margarine to give them yellow colour, scarlet red for colouring cherries etc) in the causation of hepatocellular carcinoma.

c) Naturally-occurring products. Some of the important chemical carcinogens derived from plant and microbial sources are aflatoxin B1, actinomycin D, mitomycin C, safrole and betel nuts. Out of these, aflatoxin B1 implicated in causing human hepatocellular carcinoma is the most important, especially when concomitant viral hepatitis B is present. It is derived from the fungus, *Aspergillus flavus*, that grows in stored grains and plants.

d) Miscellaneous. A variety of other chemical carcinogens having a role in the etiology of human cancer are as under:

■ **Nitrosamines and nitrosamides** are involved in gastric carcinoma. These compounds are actually made in the stomach by nitrosylation of food preservatives.

■ **Vinyl chloride monomer** derived from PVC (polyvinyl chloride) polymer in the causation of haemangiosarcoma of the liver.

■ **Asbestos** in bronchogenic carcinoma and mesothelioma, especially in smokers.

■ **Arsenical compounds** in causing epidermal hyperplasia and basal cell carcinoma.

■ **Metals** like nickel, lead, cobalt chromium etc in industrial workers causing lung cancer.

■ **Insecticides and fungicides** (e.g. aldrin, dieldrin, chlor-dane) in carcinogenesis in experimental animals.

■ **Saccharin and cyclomates** in cancer in experimental animals.

2. PROMOTER CARCINOGENS

Promoters are chemical substances which lack the intrinsic carcinogenic potential but their application subsequent to initiator exposure helps the initiated cell to proliferate further. These substances include phorbol esters, phenols, certain hormones and drugs.

i) **Phorbol esters.** The best known promoter in experimental animals is *TPA* (tetradecanoyl phorbol acetate) which acts by signal induction protein activation pathway.

ii) **Hormones.** Endogenous or exogenous oestrogen excess in promotion of cancers of endometrium and breast, prolonged administration of diethylstilbestrol in the etiology of postmenopausal endometrial carcinoma and in vaginal cancer in adolescent girls born to mothers exposed to this hormone during their pregnancy.

iii) **Miscellaneous.** e.g. dietary fat in cancer of colon, cigarette smoke and viral infections etc.

The feature of initiators and promoters are contrasted in Table 21.5.

Tests for Chemical Carcinogenicity

There are 2 main methods of testing chemical compound for its carcinogenicity:

1. EXPERIMENTAL INDUCTION. The traditional method is to administer the chemical compound under test to a batch of experimental animals like mice or other rodents by an appropriate route e.g. painting on the skin, giving orally or parenterally, or by inhalation. The chemical is administered repeatedly, the dose varied, and promoting agents are administered subsequently. After many months, the animal is autopsied and results obtained. However, all positive or negative tests cannot be applied to humans since there is sufficient species variation in susceptibility to particular carcinogen. Besides, the test is rather prolonged and expensive.

2. TESTS FOR MUTAGENICITY (AMES' TEST). A mutagen is a substance that can permanently alter the genetic composition of a cell. Ames' test evaluates the ability of a chemical to induce mutation in the mutant strain of *Salmonella typhimurium* that cannot synthesise histidine. Such strains are incubated with the potential carcinogen to which liver homogenate is added to supply enzymes required to convert procarcinogen to ultimate carcinogen. If the chemical under test is

TABLE 21.5: Contrasting Features of Initiator and Promoter Carcinogens.

FEATURE	INITIATOR	PROMOTER
1. <i>Mechanism</i>	Induction of mutation	Not mutagenic
2. <i>Dose</i>	Single for a short time	Repeated dose exposure, for a long time
3. <i>Response</i>	Sudden response	Slow response
4. <i>Change</i>	Permanent, irreversible	Change may be reversible
5. <i>Sequence</i>	Applied first, followed by promoter	Applied after prior exposure to initiator
6. <i>Effectivity</i>	Effective alone if exposed in large dose	Not effective alone
7. <i>Molecular changes</i>	Most common mutation of <i>RAS</i> oncogene, <i>TP53</i> anti-oncogene	Clonal expansion of mutated cells
8. <i>Examples</i>	Most chemical carcinogens, radiation	Hormones, phorbol esters

mutagenic, it will induce mutation in the mutant strains of *S. typhimurium* in the form of functional histidine gene, which will be reflected by the number of bacterial colonies growing on histidine-free culture medium (Fig. 21.6). Most of the carcinogenic chemicals tested positive in Ames' test are carcinogenic *in vivo*.

C. PHYSICAL CARCINOGENESIS

Physical agents in carcinogenesis are divided into 2 groups:

1. *Radiation*, both ultraviolet light and ionising radiation, is the most important physical agent. The role of radiation as carcinogenic agent is discussed below while its non-neoplastic complications are described on page 32.

2. *Non-radiation* physical agents are the various forms of injury and are less important.

1. Radiation Carcinogenesis

Ultraviolet (UV) light and ionising radiation are the two main forms of radiation carcinogens which can induce cancer in experimental animals and are implicated in causation of some forms of human cancers. A property common between the two forms of radiation carcinogens is the appearance of mutations followed by a long period of latency after initial exposure, often 10-20 years or even later. Also, radiation carcinogens may act to enhance the effect of another carcinogen (co-carcinogens) and, like chemical carcinogens, may have sequential stages of initiation and promotion in their evolution. Ultraviolet light and ionising radiation differ in their mode of action as described below:

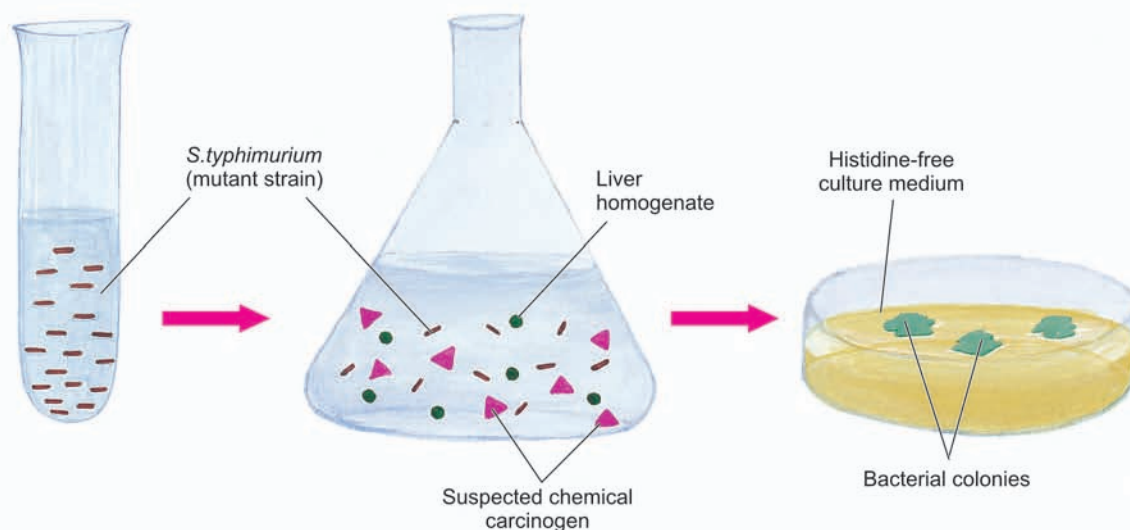


FIGURE 21.6

Schematic representation of the Ames' test.

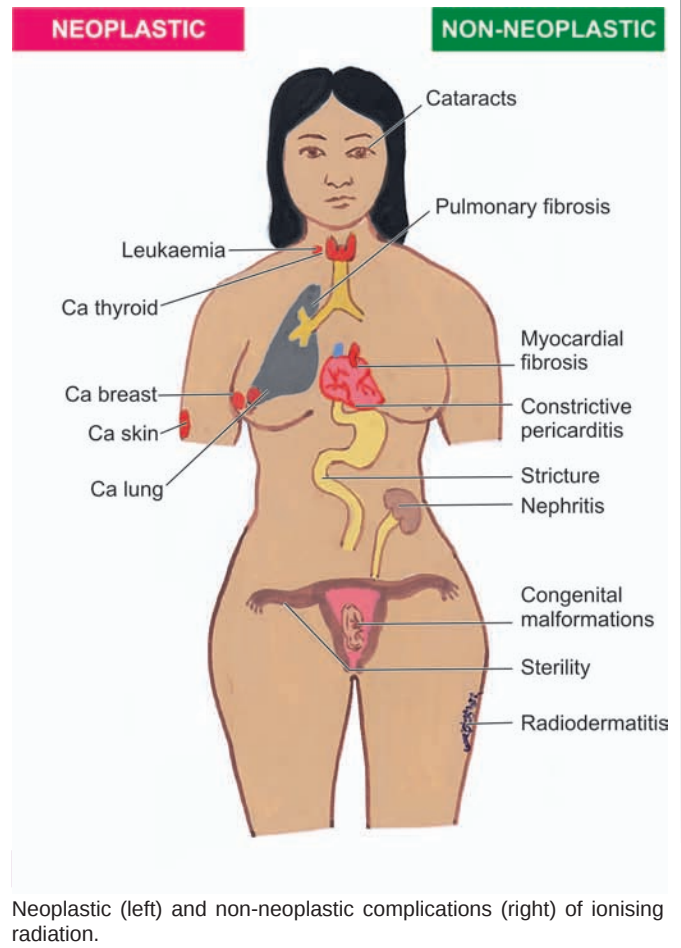
i) **ULTRAVIOLET LIGHT.** The main source of UV radiation is the sunlight; others are UV lamps and welder's arcs. UV light penetrates the skin for a few millimetres only so that its effect is limited to epidermis. The efficiency of UV light as carcinogen depends upon the extent of light-absorbing protective melanin pigmentation of the skin. In humans, excessive exposure to UV rays can cause various forms of skin cancers—squamous cell carcinoma, basal cell carcinoma and malignant melanoma. In support of this is the epidemiological evidence of high incidence of these skin cancers in fair-skinned Europeans, albinos who do not tan readily, in people in Australia and New Zealand living close to the equator who receive more sunlight, and in farmers and outdoor workers due to the effect of actinic light radiation.

Mechanism. UV radiation may have various effects on the cells. The most important is induction of mutation; others are inhibition of cell division, inactivation of enzymes and sometimes causing cell death. The most important biochemical effect of UV radiation is the formation of pyrimidine dimers in DNA. Such UV-induced DNA damage in normal individuals is repaired, while in the predisposed persons who are excessively exposed to sunlight such damage remain unrepaired. The proof in favour of mutagenic effect of UV radiation comes from following recessive hereditary diseases characterised by a defect in DNA repair mechanism and associated with high incidence of cancers:

- Xeroderma pigmentosum is predisposed to skin cancers at younger age (under 20 years of age).
- Ataxia telangiectasia is predisposed to leukaemia.
- Bloom's syndrome is predisposed to all types of cancers.
- Fanconi's anaemia with increased risk to develop cancer.

Besides, like with other carcinogens, UV radiation also induces mutated forms of oncogenes (in particular *RAS* gene) and anti-oncogenes (*TP53* gene).

ii) **IONISING RADIATION.** Ionising radiation of all kinds like X-rays, α -, β - and γ -rays, radioactive isotopes, protons and neutrons can cause cancer in animals and in man. Most frequently, radiation-induced cancers are all forms of leukaemias (except chronic lymphocytic leukaemia); others are cancers of the thyroid (most commonly papillary carcinoma), skin, breast, ovary, uterus, lung, myeloma, and salivary glands (Fig. 21.7). The risk is increased by higher dose and with high LET (linear energy transfer) such as in neutrons and



Neoplastic (left) and non-neoplastic complications (right) of ionising radiation.

α -rays than with low LET as in X-rays and γ -rays. The evidence in support of carcinogenic role of ionising radiation is cited in the following examples:

- Higher incidence of radiation dermatitis and subsequent malignant tumours of the skin was noted in X-ray workers and radiotherapists who did initial pioneering work in these fields before the advent of safety measures.
- High incidence of osteosarcoma was observed in young American watch-working girls engaged in painting the dials with luminous radium who unknowingly ingested radium while using lips to point their brushes.
- Miners in radioactive elements have higher incidence of cancers.
- Japanese atom bomb survivors of the twin cities of Hiroshima and Nagasaki after World War II have increased frequency of malignant tumours, notably acute and chronic myeloid leukaemias, and various solid tumours of breast, colon, thyroid and lung.

e) Accidental leakage at nuclear power plant in 1985 in Chernobyl (in former USSR, now in Ukraine) has caused long term hazardous effects of radioactive material to the population living in the vicinity.

f) It has been observed that therapeutic X-ray irradiation results in increased frequency of cancers, e.g. in patients of ankylosing spondylitis, in children with enlarged thymus, and in children irradiated *in utero* during investigations on the mother.

Mechanism. Radiation damages the DNA of the cell by one of the 2 possible mechanisms:

- It may directly alter the cellular DNA.
- It may dislodge ions from water and other molecules of the cell and result in formation of highly reactive free radicals that may bring about the damage.

Damage to the DNA resulting in mutagenesis is the most important action of ionising radiation. It may cause chromosomal breakage, translocation, or point mutation. The effect depends upon a number of factors such as type of radiation, dose, dose-rate, frequency and various host factors such as age, individual susceptibility, immune competence, hormonal influences and type of cells irradiated.

2. Non-radiation Physical Carcinogenesis

Mechanical injury to the tissues such as from stones in the gallbladder, stones in the urinary tract, and healed scars following burns or trauma, has been suggested as the cause of increased risk of carcinoma in these tissues but the evidence is not convincing. Asbestosis and asbestos-associated tumours of the lung include bronchogenic carcinoma and the characteristic pleural tumour is malignant mesothelioma. Other examples of physical agents in carcinogenesis are the implants of inert materials such as plastic, glass etc in prostheses or otherwise, and foreign bodies observed to cause tumour development in experimental animals. However, tumorigenesis by these materials in humans is rare.

D. BIOLOGIC CARCINOGENESIS

The epidemiological studies on different types of cancers indicate the involvement of transmissible biologic agents in their development, chiefly *viruses*. Other biologic agents implicated in carcinogenesis are as follows:

■ **Parasites** e.g. *Schistosoma haematobium* infection of the urinary bladder is associated with high incidence of squamous cell carcinoma of the urinary bladder in some parts of the world such as in Egypt. *Clonorchis sinensis*, the liver fluke, lives in the hepatic duct and is implicated in causation of cholangiocarcinoma.

■ **Fungus** *Aspergillus flavus* grows in stored grains and liberates aflatoxin; its human consumption, especially

by those with HBV infection, is associated with development of hepatocellular carcinoma.

■ **Bacteria** *Helicobacter pylori*, a gram-positive spiral-shaped micro-organism, colonises the gastric mucosa and has been found in cases of chronic gastritis and peptic ulcer; its prolonged infection may lead to gastric lymphoma and gastric carcinoma.

However, the role of viruses in the causation of cancer is more significant. It has been estimated that about 20% of all cancers worldwide are virus-associated cancers. Therefore, biologic carcinogenesis is largely *viral carcinogenesis*, described below.

VIRAL CARCINOGENESIS

The association of oncogenic viruses with neoplasia was first observed by an Italian physician Sanarelli in 1889 who noted association between myxomatosis of rabbits with poxvirus. The contagious nature of the common human wart was first established in 1907. Since then, a number of viruses capable of inducing tumours (oncogenic viruses) in experimental animals, and some implicated in man, have been identified.

Oncogenic viruses can be transmitted by one of the 3 routes:

- Vertical transmission*, when the infection is genetically transmitted from infected parents to offsprings.
- Horizontal transmission*, when the infection passes from one to another by direct contact as occurs in most contagious diseases.
- By inoculation* as is done in experimental animals.

Based on their nucleic acid content, oncogenic viruses fall into 2 broad groups:

- Those containing deoxyribonucleic acid are called *DNA oncogenic viruses*.
- Those containing ribonucleic acid are termed *RNA oncogenic viruses*.

The two types of oncogenic viruses are described separately below, followed by their mechanisms of action. Most of the work is based on studies in experimental animals and only some have a role in human neoplasia.

DNA Oncogenic Viruses

DNA oncogenic viruses have direct access to the host cell nucleus and are incorporated into the genome of the host cell. DNA viruses are classified into 5 subgroups, each of which is capable of producing neoplasms in different hosts (Table 21.6). These are: Papovaviruses, Herpesviruses, Adenoviruses, Poxviruses and Hepadna viruses.

1. PAPOVAVIRUSES. This group consists of the papilloma virus including the human papilloma virus

TABLE 21.6: DNA Oncogenic Viruses.

VIRUS	HOST	ASSOCIATED TUMOUR
1. PAPOVAVIRUSES		
<i>Human papilloma virus</i>	Humans	Cervical cancer and its precursor lesions, squamous cell carcinoma at other sites Skin cancer in epidermodysplasia verruciformis Papillomas (warts) on skin, larynx, genitals (genital warts)
<i>Papilloma viruses</i>	Cotton-tail rabbits Bovine	Papillomas (Warts) Alimentary tract cancer
<i>Polyoma virus</i>	Mice	Various carcinomas, sarcomas
<i>SV-40 virus</i>	Monkeys Hamsters Humans	Harmless Sarcoma ? Mesothelioma
2. HERPESVIRUSES		
<i>Epstein-Barr virus</i>	Humans	Burkitt's lymphoma Nasopharyngeal carcinoma
<i>Human herpesvirus 8</i> (<i>Kaposi's sarcoma herpesvirus</i>)	Humans	Kaposi's sarcoma B cell lymphoma
<i>Lucke' frog virus</i>	Frog	Renal cell carcinoma
<i>Marek's disease virus</i>	Chickens	T-cell leukaemia-lymphoma
3. ADENOVIRUSES		
	Hamsters	Sarcomas
4. POXVIRUSES		
	Rabbits	Myxomatosis
	Humans	Molluscum contagiosum, papilloma
5. HEPADNAVIRUSES		
<i>Hepatitis B virus</i>	Humans	Hepatocellular carcinoma

(HPV), polyoma virus and SV-40 (simian vacuolating) virus. These viruses have an etiologic role in a variety of benign and malignant neoplasms in animals and in humans:

i) Papilloma viruses. These viruses were the first to be implicated in the etiology of any human neoplasia. These viruses appear to replicate in the layers of stratified squamous epithelium. About 70 types of HPV have been identified, different types being implicated in cutaneous warts (benign squamous papillomas), genital warts (condyloma acuminata) and in squamous cell cancers. The following examples are cited to demonstrate their role in oncogenesis:

In humans—

■ HPV was first detected as etiologic agent in common *skin warts* (squamous cell papillomas) by Shope in 1933; the condition is infectious. Current evidence supports implication of low risk HPV types 1, 2, 4 and 7.

■ Viral DNA of high risk HPV types 16 and 18 has been seen in 75-100% cases of *invasive cervical cancer* and its precursor lesions (carcinoma *in situ* and dysplasia) and is strongly implicated.

■ HPV types 6 and 11 are involved in the etiology of genital warts or *condyloma acuminata*.

■ HPVs are also involved in causation of *other squamous cell carcinomas* such as of anus, perianal region, vulva, penis and oral cavity.

■ HPV is responsible for causing an uncommon condition, *epidermodysplasia verruciformis*. The condition is characterised by multiple skin warts and a genetic defect in the cell-mediated immunity. About one third of cases develop squamous cell carcinoma in the sun-exposed warts.

■ Some strains of HPV are responsible for causing multiple *juvenile papillomas* of the larynx.

In animals—

■ Benign warty lesions similar to those seen in humans are produced by different members of the papilloma virus family in susceptible animals such as in rabbits by cottontail rabbit papilloma virus, and in cattle by bovine papilloma virus (BPV).

■ There is evidence to suggest the association of BPV and cancer of the alimentary tract in cattle.

ii) Polyoma virus. Polyoma virus occurs as a natural infection in mice.

■ *In animals*—Polyoma virus infection is responsible for various kinds of carcinomas and sarcomas in immunodeficient (nude) mice and other rodents.

■ *In humans*—Polyoma virus infection is not known to produce any human tumour but can cause progressive demyelinating leucoencephalopathy which is a fatal demyelinating disease.

iii) SV-40 virus. As the name suggests, simian vacuolating virus occurs in monkeys without causing any harm but can induce sarcoma in hamsters.

Recently, there is evidence of involvement of SV-40 infection in mesothelioma of the pleura.

2. HERPESVIRUSES. Primary infection of all the herpesviruses in man persists probably for life in a latent stage which can get reactivated later. Important members of herpesvirus family are Epstein-Barr virus, herpes simplex virus type 2 (HSV-2) and human herpesvirus 8 (HHV8), cytomegalovirus (CMV), Lucke's frog virus and Marek's disease virus. Out of these, Lucke's frog virus and Marek's disease virus are implicated in animal tumours only (renal cell carcinoma and T-cell leukaemia-lymphoma respectively). Earlier oncogenic role assigned to HSV-2 and CMV in human tumours has now been refuted. The other two—EBV and HHV are implicated in human tumours as follows.

EPSTEIN-BARR VIRUS (EBV). EBV infects human B-lymphocytes and stimulates them to proliferate. EBV is implicated in the following human tumours—Burkitt's lymphoma, anaplastic nasopharyngeal carcinoma, post-transplant lymphoproliferative disease, primary CNS lymphoma in AIDS patients, and Hodgkin's lymphoma. It is also shown to be the cause of infectious mononucleosis, a self-limiting disease in humans. The role of EBV in the first two human tumours is given below while others find mention elsewhere in relevant chapters.

Burkitt's lymphoma. Burkitt's lymphoma was initially noticed in African children by Burkitt in 1958 but is now known to occur in 2 forms—*African endemic form*, and *sporadic form* seen elsewhere in the world. The morphological aspects of the tumour are explained on page 530, while oncogenesis is described here.

There is strong evidence linking Burkitt's lymphoma, a B-lymphocyte neoplasm, with EBV as observed from the following features:

- Over 90% of Burkitt's lymphomas are EBV-positive in which the tumour cells carry the viral DNA.
- 100% cases of Burkitt's lymphoma show elevated levels of antibody titers to various EBV antigens.
- EBV has strong tropism for B lymphocytes. EBV-infected B cells grown in cultures are immortalised i.e.

they continue to develop further along B cell-line to propagate their progeny in the altered form.

d) Though EBV infection is almost worldwide in all adults and is also known to cause self-limiting infectious mononucleosis, but the fraction of EBV-infected circulating B cells in such individuals is extremely small.

e) Linkage between Burkitt's lymphoma and EBV infection is very high in African endemic form of the disease and probably in cases of AIDS than in sporadic form of the disease.

However, a few observations, especially regarding sporadic cases of Burkitt's lymphoma, suggest that certain other supportive factors may be contributing. *Immunosuppression* appears to be one such most significant factor. The evidence in favour is as follows:

■ The normal EBV-infected individuals as well as cases developing infectious mononucleosis are able to mount good immune response so that they do not develop Burkitt's lymphoma.

■ In immunosuppressed patients such as in HIV infection and organ transplant recipients, there is marked reduction in body's T-cell immune response and higher incidence of this neoplasm.

■ It is observed that malaria, which confers immunosuppressive effect on the host, is prevalent in endemic proportions in regions where endemic form of Burkitt's lymphoma is frequent. This supports the linkage of EBV infection and immunosuppression in the etiology of Burkitt's lymphoma.

Anaplastic nasopharyngeal carcinoma. This is the other tumour having close association with EBV infection. The tumour is prevalent in South-East Asia, especially in the Chinese, and in Eskimos. The morphology of nasopharyngeal carcinoma is described in Chapter 16. The evidence linking EBV infection with this tumour is as follows:

- 100% cases of nasopharyngeal carcinoma carry DNA of EBV in tumour cells.
- Individuals with this tumour have high titers of antibodies to various EBV antigens.

However, like in case of Burkitt's lymphoma, there may be some *co-factors* such as genetic susceptibility that account for the unusual geographic distribution.

HUMAN HERPESVIRUS 8 (HHV 8). It has been shown that infection with HHV 8 or Kaposi's sarcoma-associated herpesvirus (KSHV) is associated with Kaposi's sarcoma, a vascular neoplasm common in patients of AIDS. Compared to sporadic Kaposi's sarcoma, the AIDS-associated tumour is multicentric and more aggressive. HHV 8 has lymphotropism and is also

implicated in causation of B cell lymphoma and multicentric variant of Castleman's disease.

3. ADENOVIRUSES. The human adenoviruses cause upper respiratory infections and pharyngitis.

■ *In humans*, they are not known to be involved in any tumour.

■ *In hamsters*, they may induce sarcomas.

4. POXVIRUSES. This group of oncogenic viruses is involved in the etiology of following lesions:

■ *In rabbits*—poxviruses cause myxomatosis.

■ *In humans*—poxviruses cause molluscum contagiosum and may induce squamous cell papilloma.

5. HEPADNAVIRUSES. *Hepatitis B virus (HBV)* is a member of hepadnavirus family. HBV infection in man causes an acute hepatitis and is responsible for a carrier state, which can result in some cases to chronic hepatitis progressing to hepatic cirrhosis, and onto hepatocellular carcinoma. These lesions and the structure of HBV are described in detail in Chapter 28. Suffice this to say here that there is strong epidemiological evidence linking HBV infection to development of hepatocellular carcinoma as evidenced by the following:

a) The geographic differences in the incidence of hepatocellular carcinoma closely match the variation in prevalence of HBV infection e.g. high incidence in Far-East and Africa.

b) Epidemiological studies in high incidence regions indicate about 200 times higher risk of developing hepatocellular carcinoma in HBV-infected cases as compared to uninfected population in the same area.

Possible mechanism of hepatocellular carcinoma occurring in those harbouring long standing infection with HBV is chronic destruction of HBV-infected

hepatocytes followed by continued hepatocyte proliferation. This process renders the hepatocytes vulnerable to the action of other risk factors such as to aflatoxin causing mutation and neoplastic proliferation.

More recent evidence has assigned an oncogenic role to another hepatotropic virus, hepatitis C virus (HCV), an RNA virus unrelated to HBV. HCV is implicated in about half the cases of hepatocellular carcinoma in much the same way as HBV.

RNA Oncogenic Viruses

RNA oncogenic viruses are retroviruses i.e. they contain the enzyme reverse transcriptase (RT), though all retroviruses are not oncogenic (Table 21.7). The enzyme, reverse transcriptase, is required for reverse transcription of viral RNA to synthesise viral DNA strands i.e. reverse of normal—rather than DNA encoding for RNA synthesis, viral RNA transcripts for the DNA by the enzyme RT present in the RNA viruses. RT is a DNA polymerase and helps to form complementary DNA (cDNA) that moves in to host cell nucleus and gets incorporated into it.

Based on their activity to transform target cells into neoplastic cells, RNA viruses are divided into 3 sub-groups—acute transforming viruses, slow transforming viruses, and human T-cell lymphotropic viruses (HTLV). The former two are implicated in inducing a variety of tumours in animals while HTLV is causative for human T-cell leukaemia and lymphoma.

In general, RNA viruses have 3 genes: *gag* gene (for group antigen), *pol* gene (that codes for polymerase enzyme), and *env* gene (for envelop protein), while acute transforming viruses have one more fourth gene *tat* gene that transforms the oncogene in the susceptible cell.

TABLE 21.7: RNA Oncogenic Viruses.

VIRUS	HOST	ASSOCIATED TUMOUR
1. ACUTE TRANSFORMING VIRUSES		
<i>Rous sarcoma virus</i>	Chickens	Sarcoma
<i>Leukaemia-sarcoma virus</i>	Avian, feline, bovine, primate	Leukaemias, sarcomas
2. SLOW TRANSFORMING VIRUSES		
<i>Mouse mammary tumour virus</i> (Bittner milk factor)	Mice, cats, bovine Daughter mice	Leukaemias, lymphomas Breast cancer
3. HUMAN T-CELL LYMPHOTROPIC VIRUS (HTLV)		
<i>HTLV-I</i>	Human	Adult T-cell leukaemia lymphoma (ATLL)
<i>HTLV-II</i>	Human	T-cell variant of hairy cell leukaemia
4. HEPATITIS C VIRUS		
<i>HCV</i>	Human	Hepatocellular carcinoma

1. ACUTE TRANSFORMING VIRUSES. This group includes retroviruses which transform all the cells infected by them into malignant cells rapidly ('acute'). All the viruses in this group possess one or more viral oncogenes (*v-oncs*). All the members of acute transforming viruses discovered so far are defective viruses in which the particular *v-onc* has substituted other essential genetic material such as *gag*, *pol* and *env*. These defective viruses cannot replicate by themselves unless the host cell is infected by another 'helper virus'. Acute oncogenic viruses have not been detected in any human tumour so far, though they have been identified in tumours in different animals, e.g.

- a) Rous sarcoma virus in chickens.
- b) Leukaemia-sarcoma viruses of various types such as avian, feline, bovine and primate.

2. SLOW TRANSFORMING VIRUSES. These oncogenic retroviruses cause development of leukaemias and lymphomas in different species of animals (e.g. in mice, cats and bovine) and include the mouse mammary tumour virus (MMTV) that causes breast cancer in the daughter-mice suckled by the MMTV-infected mother via the causal agent in the mother's milk (*Bittner milk factor*). These viruses have long incubation period between infection and development of neoplastic transformation ('slow'). Slow transforming viruses cause neoplastic transformation by *insertional mutagenesis* i.e. viral DNA synthesised by viral RNA via reverse transcriptase is *inserted* or integrated near the proto-oncogenes of the host cell resulting in enhanced expression of proto-oncogenes as well as causes genetic damage (*mutagenesis*) to the host cell genome leading to neoplastic transformation.

3. HUMAN T-CELL LYMPHOTROPIC VIRUSES (HTLV). HTLV is a form of slow transforming virus but is described separately because of 2 reasons:

- i) This is the only retrovirus implicated in human cancer.
- ii) The mechanism of neoplastic transformation is different from slow transforming as well as from acute transforming viruses.

Four types of HTLVs are recognised—HTLV-I, HTLV-II, HTLV-III and HTLV-IV. It may be mentioned in passing here that the etiologic agent for AIDS, HIV, is also an HTLV (HTLV-III) as described in Chapter 13.

A link between HTLV-I infection and adult T-cell leukaemia-lymphoma syndrome (ATLL) has been identified while HTLV-II is implicated in causation of T-cell variant of hairy cell leukaemia. HTLV-I is transmitted through sexual contact, by blood, or to infants during

breast feeding. The highlights of this association and mode of neoplastic transformation are as under:

- i) Epidemiological studies by tests for antibodies have shown that HTLV-I infection is endemic in parts of Japan and West Indies where the incidence of ATLL is high. The latent period after HTLV-I infection is, however, very long (20-30 years).
- ii) The initiation of neoplastic process is similar to that for Burkitt's lymphoma except that HTLV-I has tropism for CD4+T lymphocytes as in HIV infection, while EBV of Burkitt's lymphoma has tropism for B lymphocytes.
- iii) As in Burkitt's lymphoma, immunosuppression plays a supportive role in the neoplastic transformation by HTLV-I infection.

The underlying *molecular mechanism* of neoplastic transformation by HTLV-I infection differs from acute transforming viruses because it does not contain *v-onc*, and from other slow transforming viruses because it does not have fixed site of insertion for insertional mutagenesis.

The genome of HTLV-I contains *gag*, *pol* and *env* genes similar to other retroviruses but in addition it contains *tat* gene region also which stimulates neoplastic cell proliferation of T cells. Initially, this proliferation of T cells is polyclonal which then undergoes new mutations and leads to monoclonal T cell leukaemia-lymphoma. Chronic leukaemia retrovirus, HTLV-II, brings about transformation by 'promoter insertion' that produces oncogenesis by oncogenes and growth factors.

Mechanisms of Viral Oncogenesis

Mode of oncogenesis by DNA and RNA oncogenic viruses are different and are illustrated schematically in Fig. 21.8 and 21.9 respectively.

1. Mode of DNA viral oncogenesis. Host cells infected by DNA oncogenic viruses may have one of the following 2 results (Fig. 21.8):

- i) *Replication.* The virus may replicate in the host cell with consequent lysis of the infected cell and release of virions.
- ii) *Integration.* The viral DNA may integrate into the host cell DNA.

The latter event (integration) results in neoplastic transformation of the host cell, while the former (replication) brings about cell death but no neoplastic transformation. A feature essential for host cell transformation is the expression of virus-specific T-(transforming protein) antigens immediately after infection of the host cell by DNA oncogenic virus.

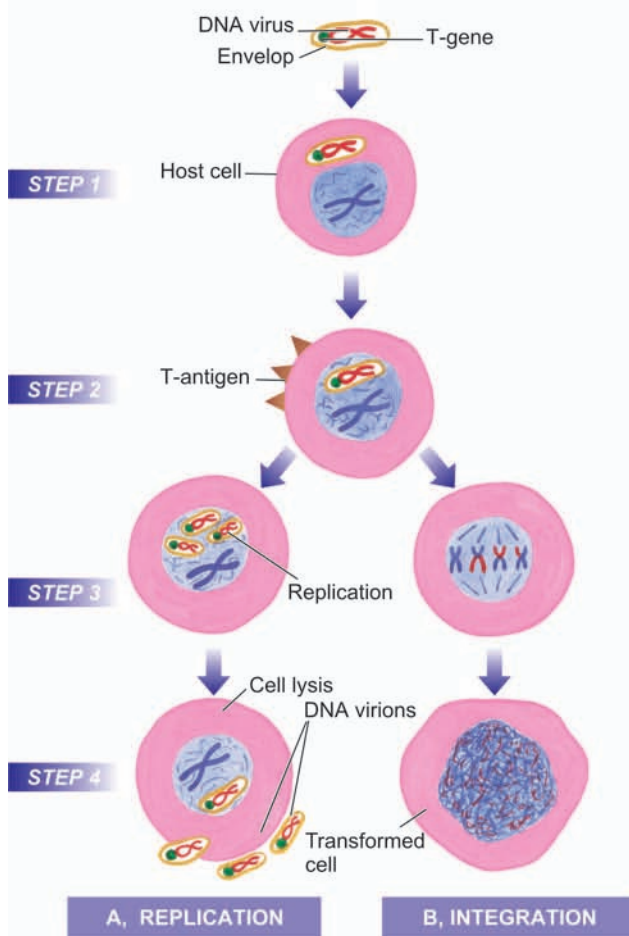


FIGURE 21.8

Replication and integration of DNA virus in the host cell.

A, Replication: *Step 1.* The DNA virus invades the host cell. *Step 2.* Viral DNA is incorporated into the host nucleus and T-antigen is expressed immediately after infection. *Step 3.* Replication of viral DNA occurs and other components of virion are formed. The new virions are assembled in the cell nucleus. *Step 4.* The new virions are released, accompanied by host cell lysis. **B, Integration:** *Steps 1 and 2* are similar as in replication. *Step 3.* Integration of viral genome into the host cell genome occurs which requires essential presence of functional T-antigen. *Step 4.* A 'transformed (neoplastic) cell' is formed.

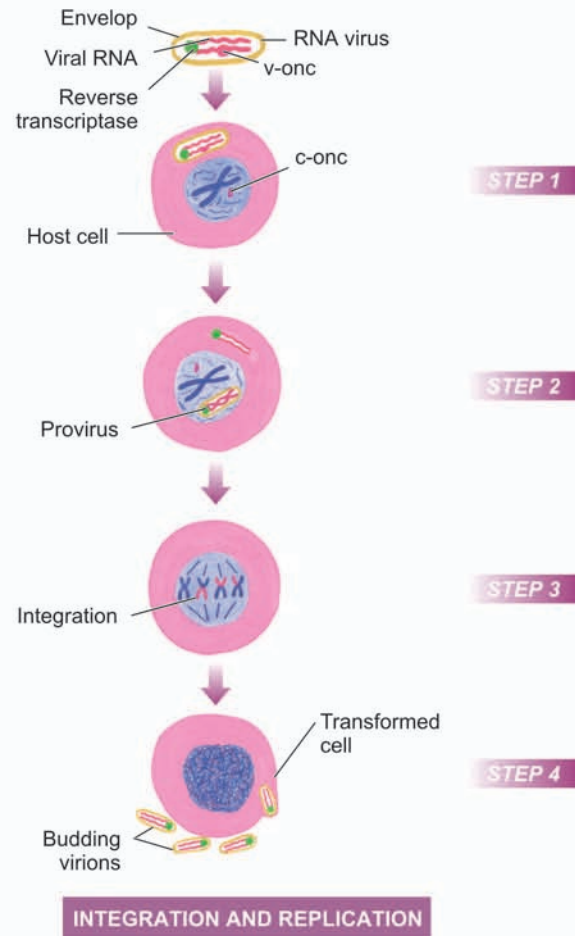


FIGURE 21.9

Integration and replication of RNA virus (retrovirus) in the host cell.

Step 1. The RNA virus invades the host cell. The viral envelope fuses with the plasma membrane of the host cell; viral RNA genome as well as reverse transcriptase are released into the cytosol. *Step 2.* Reverse transcriptase acts as template to synthesise single strand of matching viral DNA which is then copied to form complementary DNA resulting in double-stranded viral DNA (provirus). *Step 3.* The provirus is integrated into the host cell genome producing 'transformed host cell.' *Step 4.* Integration of the provirus brings about replication of viral components which are then assembled and released by budding.

2. Mode of RNA viral oncogenesis. As the name suggests, RNA viruses or retroviruses contain two identical strands of RNA and the enzyme, reverse transcriptase. The steps involved in transformation of host cells by RNA oncogenic viruses are as under (Fig. 21.9):

- Reverse transcriptase is RNA-dependent DNA synthetase that acts as a template to synthesise a single strand of matching viral DNA i.e. reverse of the normal in which DNA is transcribed into messenger RNA.

- The single strand of viral DNA is then copied by DNA dependent DNA synthetase to form another strand of complementary DNA resulting in double-stranded viral DNA or provirus.
- The provirus is then integrated into the DNA of the host cell genome and may transform the cell into neoplastic cell.
- Retroviruses are replication-competent. The host cells which allow replication of integrated retrovirus are

called permissive cells. Non-permissible cells do not permit replication of the integrated retrovirus.

v) Viral replication begins after integration of the provirus into host cell genome. Integration results in transcription of proviral genes or progenes into messenger RNA which then forms *components of the virus particle*—virion core protein from *gag* gene, reverse transcriptase from *pol* gene, and envelope glycoprotein from *env* gene. The three components of virus particle are then assembled at the plasma membrane of the host cell and the virus particles released by budding off from the plasma membrane, thus completing the process of replication.

VIRUSES AND HUMAN CANCER: A SUMMARY

In man, epidemiological as well as circumstantial evidence has been accumulating since the discovery of contagious nature of common human wart (papilloma) in 1907 that cancer may have viral etiology. Presently, about 20% of all human cancers worldwide are believed to have 'virus association'. Aside from experimental evidence, the etiologic role of DNA and RNA viruses in a variety of human neoplasms has already been explained above. Here, a summary of different viruses implicated in human tumours is presented (Fig. 21.10):

Benign tumours. There are 2 conditions which are actually doubtful as tumours in which definite viral etiology is established. These are:

- i) Human wart (papilloma) caused by human papilloma virus; and
- ii) Molluscum contagiosum caused by poxvirus.

Malignant tumours. The following 8 human cancers have enough epidemiological, serological, and in some cases genomic evidence that viruses are implicated in their etiology:

- i) *Burkitt's lymphoma* by Epstein-Barr virus.
- ii) *Nasopharyngeal carcinoma* by Epstein-Barr virus.
- iii) *Primary hepatocellular carcinoma* by hepatitis B virus and hepatitis C virus.

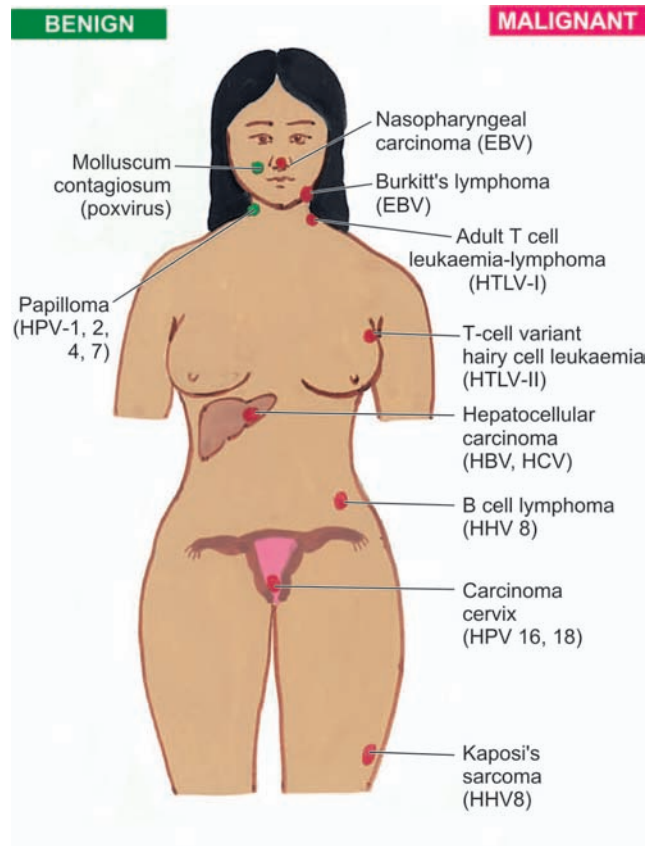


FIGURE 21.10

Viruses (in brackets) in human tumours.

iv) *Cervical cancer* by high risk human papilloma virus types (HPV 16 and 18).

v) *Kaposi's sarcoma* by human herpes virus type 8 (HHV 8).

vi) *B cell lymphoma* by HHV8.

vii) *Adult T-cell leukaemia and lymphoma* by HTLV-I.

viii) *T-cell variant of hairy cell leukaemia* by HTLV-II.



Two major aspects of clinical significance in assessing the course and management of neoplasia are: tumour-host inter-relationship (i.e. the effect of tumour on host and *vice versa*) and laboratory diagnosis of cancer.

TUMOUR-HOST INTER-RELATIONSHIP

The natural history of a neoplasm depends upon 2 features:

- i) Effect of tumour on host
- ii) Host response against tumour (Immunology of cancer)

EFFECT OF TUMOUR ON HOST

Malignant tumours produce more ill-effects than the benign tumours. The effects may be local, or generalised and more widespread.

1. LOCAL EFFECTS. Both benign and malignant tumours cause local effects on the host due to their size or location. Malignant tumours due to rapid and invasive growth potential have more serious effects. Some of the local effects of tumours are as under:

i) Compression. Many benign tumours pose only a cosmetic problem. Some benign tumours, however, due to their critical location, have more serious consequences e.g. pituitary adenoma may lead to serious endocrinopathy; a small benign tumour in ampulla of Vater may lead to biliary obstruction.

ii) Mechanical obstruction. Benign and malignant tumours in the gut may produce intestinal obstruction.

iii) Tissue destruction. Malignant tumours, both primary and metastatic, infiltrate and destroy the vital structures.

iv) Infarction, ulceration, haemorrhage. Cancers have a greater tendency to undergo infarction, surface ulceration and haemorrhage than the benign tumours. Secondary bacterial infection may supervene. Large tumours in mobile organs (e.g. an ovarian tumour) may undergo torsion and produce infarction and haemorrhage.

2. CANCER CACHEXIA. Patients with advanced and disseminated cancers terminally have asthenia (emaciation), and anorexia, together referred to as cancer

cachexia (meaning wasting). Exact mechanism of cachexia is not clear but it does not occur due to increased nutritional demands of the tumour. Possibly, cachectin or tumour necrosis factor α (TNF- α) and interleukin-1 derived from macrophages play a contributory role in cachexia. The various other causes include necrosis, ulceration, haemorrhage, infection, malabsorption, anxiety, pain, insomnia, hyper-metabolism and pyrexia.

3. FEVER. Fever of unexplained origin may be presenting feature in some malignancies such as in Hodgkin's disease, adenocarcinoma kidney, osteogenic sarcoma and many other tumours. The exact mechanism of tumour associated fever is not known but probably the tumour cells themselves elaborate pyrogens.

4. PARANEOPLASTIC SYNDROMES. Paraneoplastic syndromes (PNS) are a group of conditions developing in patients with advanced cancer which are neither explained by direct and distant spread of the tumour, nor by the usual hormone elaboration by the tissue of origin of the tumour. About 10 to 15% of the patients with advanced cancer develop one or more of the syndromes included in the PNS. Rarely, PNS may be the earliest manifestation of a latent cancer.

The various clinical syndromes included in the PNS are as summarised in Table 22.1 and are briefly outlined below:

i) Endocrine syndrome. Elaboration of hormones or hormone-like substances by cancer cells of non-endocrine origin is called as ectopic hormone production. Some examples are given below:

a) *Hypercalcaemia.* Symptomatic hypercalcaemia unrelated to hyperparathyroidism is the most common syndrome in PNS. It occurs from elaboration of parathormone-like substance by tumours such as squamous cell carcinoma of the lung, carcinoma kidney, breast and adult T cell leukaemia lymphoma.

b) *Cushing's syndrome* About 10% patients of small cell carcinoma of the lung elaborate ACTH or ACTH-like substance producing Cushing's syndrome. In addition, cases with pancreatic carcinoma and neurogenic tumours may be associated with Cushing's syndrome.

TABLE 22.1: Summary of Paraneoplastic Syndromes.

CLINICAL SYNDROME	UNDERLYING CANCER	MECHANISM
1. ENDOCRINE SYNDROME:		
i. <i>Hypercalcaemia</i>	Lung (sq. cell ca.), kidney, breast, Adult T-cell leukaemia-lymphoma	Parathromone-like protein vitamin D
ii. <i>Cushing's syndrome</i>	Lung (small cell carcinoma), pancreas, neural tumours	ACTH or ACTH-like substance
iii. <i>Inappropriate anti-diuresis</i>	Lung (small cell ca.), prostate, intracranial tumour	ADH or atrial natriuretic factor
iv. <i>Hypoglycaemia</i>	Pancreas (islet cell tumour), mesothelioma, fibrosarcoma	Insulin or insulin-like substance
v. <i>Carcinoid syndrome</i>	Bronchial carcinoid tumour, carcinoma pancreas, stomach	Serotonin, bradykinin
vi. <i>Polycythaemia</i>	Kidney, liver, cerebellar haemangioma	Erythropoietin
2. NEUROMUSCULAR SYNDROMES:		
i. <i>Myasthenia gravis</i>	Thymoma	Immunologic
ii. <i>Neuromuscular disorders</i>	Lung (small cell ca.), breast	Immunologic
3. OSSEOUS, JOINT AND SOFT TISSUE:		
i. <i>Hypertrophic osteoarthropathy</i>	Lung	Not known
ii. <i>Clubbing of fingers</i>	Lung	Not known
4. HAEMATOLOGIC SYNDROMES:		
i. <i>Thrombophlebitis</i> (Trousseau's phenomenon)	Pancreas, lung, GIT	Hypercoagulability
ii. <i>Non-bacterial thrombotic endocarditis</i>	Advanced cancers	Hypercoagulability
iii. <i>Disseminated intravascular coagulation (DIC)</i>	AML, adenocarcinoma	Chronic thrombotic phenomena
iv. <i>Anaemia</i>	Thymoma	Unknown
5. GASTROINTESTINAL SYNDROMES:		
i. <i>Malabsorption</i>	Lymphoma of small bowel	Hypoalbuminaemia
6. RENAL SYNDROMES:		
i. <i>Nephrotic syndrome</i>	Advanced cancers	Renal vein thrombosis, systemic amyloidosis
7. CUTANEOUS SYNDROMES:		
i. <i>Acanthosis nigricans</i>	Stomach, large bowel	Immunologic
ii. <i>Seborrheic keratosis</i>	Bowel	Immunologic
iii. <i>Exfoliative dermatitis</i>	Lymphoma	Immunologic
8. AMYLOIDOSIS:		
i. <i>Primary</i>	Multiple myeloma	Immunologic (AL protein)
ii. <i>Secondary</i>	Kidney, lymphoma, solid tumours	AA protein

c) *Polycythaemia*. Secretion of erythropoietin by certain tumours such as renal cell carcinoma, hepatocellular carcinoma and cerebellar haemangioma may cause polycythaemia.

d) *Hypoglycaemia*. Elaboration of insulin-like substance by fibrosarcomas, islet cell tumours of pancreas and mesothelioma may cause hypoglycaemia.

ii) Neuromyopathic syndromes. About 5% of cancers are associated with progressive destruction of neurons throughout the nervous system without evidence of metastasis in the brain and spinal cord. This is probably mediated by immunologic mechanisms. The changes in the neurons may affect the muscles as well. The changes are: peripheral neuropathy, cortical cerebellar degeneration, myasthenia gravis syndrome, polymyositis.

iii) Effects on osseous, joints and soft tissue. e.g. hypertrophic osteoarthropathy and clubbing of fingers in cases of bronchogenic carcinoma by unknown mechanism.

iv) Haematologic and vascular syndrome. e.g. venous thrombosis (Trousseau's phenomenon), non-bacterial thrombotic endocarditis, disseminated intravascular coagulation (DIC), leukemoid reaction and normocytic normochromic anaemia occurring in advanced cancers. Autoimmune haemolytic anaemia may be associated with B-cell tumours.

v) Gastrointestinal syndromes. Malabsorption of various dietary components as well as hypoalbuminaemia may be associated with a variety of cancers which do not directly involve small bowel.

vi) Renal syndromes. Renal vein thrombosis or systemic amyloidosis may produce nephrotic syndrome in patients with cancer.

vii) Cutaneous syndromes. Acanthosis nigricans characterised by the appearance of black warty lesions in the axillae and the groins may appear in the course of adenocarcinoma of gastrointestinal tract. Other cutaneous lesions in PNS include seborrheic dermatitis in advanced malignant tumours and exfoliative dermatitis in lymphomas and Hodgkin's disease.

viii) Amyloidosis. Primary amyloid deposits may occur in multiple myeloma whereas renal cell carcinoma and other solid tumours may be associated with secondary systemic amyloidosis.

HOST RESPONSE AGAINST TUMOUR (IMMUNE SURVEILLANCE OF CANCER)

It has long been thought that host defense mechanism in the form of immunological response exists so as to counter the growth and spread of cancer, *albeit*, more often partially. The following observations provide basis for this thinking:

1. Certain cancers evoke significant lymphocytic infiltrates composed of immunocompetent cells and such tumours have somewhat better prognosis e.g. medullary carcinoma breast, seminoma testis.
2. Rarely, a cancer may spontaneously regress partially or completely, probably under the influence of host defense mechanism. One such example is rare spontaneous disappearance of malignant melanoma from the primary site only to reappear as metastasis.
3. It is highly unusual to have primary and secondary tumours in the spleen due to its ability to destroy the growth and proliferation of tumour cells.
4. Immune surveillance exists is substantiated by increased frequency of cancers in immunodeficient host e.g. in AIDS patients, or in organ transplant recipients.

In an attempt to substantiate the above observations and to understand the underlying host defense mechanisms, experimental animal studies involving tumour transplants were carried out. The findings of animal experiments coupled with research on human cancers has led to the concept of immunology of cancer described below under the following headings:

1. Tumour antigens
2. Immune responses
3. Prospects of immunotherapy

1. TUMOUR ANTIGENS. It has been observed from experimental animal studies and in humans that following two types of tumour antigens encountered by cytotoxic T lymphocytes CTL, also called CD8+T cells:

i) Tumour-specific antigens (TSA) are located on tumour cells but are not present on normal cells. They are unique or specific antigens for particular tumour and not shared by normal cells. Therefore, TSAs are targets of attack by tumour-specific cytotoxic CD8+T lymphocytes. The examples are:

- a) Testis specific antigen having MAGE genes.
- b) Antigens responsible for mutational change in some tumours e.g. *TP53*, *RAS* gene, β -catenin etc.
- c) Overexpressed antigens e.g. *HER2* protein in cancer of the breast, ovary.
- d) Antigens from oncogenic viruses e.g. HPV, EBV.
- e) Mucins associated antigens e.g. in cancers of the pancreas, breast, ovary.
- f) Oncofoetal antigens e.g. α -foetoprotein, carcino-embryonic antigen in cancers of liver, colon.

ii) Tumour associated antigen (TAA) are present on tumour cells as well as on some normal cells. TAA are, thus, antigens shared by tumour cells and normal host cells from where the tumour originated. These antigens represent the stage at which the differentiation of tumour cells is arrested. The examples in this category are:

- a) Tissue specific antigens such as melanocyte specific proteins.
- b) Differentiation specific antigens e.g. CD10 in benign prostatic epithelium, prostate specific antigen (PSA) in carcinoma of prostate.

2. IMMUNE RESPONSES. The nature of host immune response to tumours demonstrated in animal studies and in patients with various types of cancers can be categorised as under:

- i) Cell-mediated mechanism
- ii) Humoral mechanism
- iii) Inhibitory (regulatory) mechanism.

i) Cell-mediated mechanism. This is the main mechanism of destruction of tumour cells by the host. The following cellular responses can destroy the tumour cells and induce tumour immunity in humans:

a) *Specifically sensitised cytotoxic T lymphocytes (CTL)* i.e. CD8+ T cells which are directly cytotoxic requiring contact between them and tumour cell. They specifically attack the virally induced cancers e.g. in Burkitt's lymphoma (EBV-induced), invasive squamous cell carcinoma (HPV-induced).

b) *Natural killer (NK) cells* are lymphocytes which destroy tumour cells without sensitisation, either directly or by antibody-dependent cellular cytotoxicity (ADCC). They are the first line of defense against tumour cells.

c) *Macrophages* are activated by interferon- γ secreted by T-cells and NK-cells, and therefore there is close collaboration of these lymphocytes with macrophages. Activated macrophages mediate cytotoxicity by production of oxygen free radicals or by tumour necrosis factor.

ii) Humoral mechanism. Humoral antibodies may act by complement activation or by antibody-dependent cytotoxicity by NK cells.

iii) Inhibitory (Regulatory) mechanism. In spite of host immune responses, most cancers grow relentlessly. This is due to some of the following controlling mechanisms:

a) During progression of the cancer, immunogenic cells may disappear.

b) Cytotoxic T-cells and NK-cells may play a self regulatory role.

c) Immunosuppression mediated by various acquired carcinogenic agents (viruses, chemicals, radiation).

d) Immunoinhibitory role of factors secreted by tumours e.g. transforming growth factor- β .

The mechanisms of these immune responses are schematically illustrated in Fig. 22.1.

3. PROSPECTS OF IMMUNOTHERAPY. Despite the existence of anti-tumour immune responses, the cancers still progress and eventually cause death of the host. The immune responses to be effective enough must eliminate the tumour cells more rapidly than their rate of proliferation and hence the role of boosting the immune response or immunotherapy.

i) Non-specific stimulation of the host immune response was initially attempted with BCG, *Corynebacterium parvum* and levamisole, but except slight effect in acute lymphoid leukaemia, it failed to have any significant influence in any other tumour.

ii) Specific stimulation of the immune system was attempted next by immunising the host with irradiated tumour cells but failed to yield desired results because

if the patient's tumour within the body failed to stimulate effective immunity, the implanted cells of the same tumour are unlikely to do so.

iii) Current status of immunotherapy is focussed on following three main approaches:

a) *Cellular immunotherapy* consists of infusion of tumour-specific cytotoxic T cells which will increase the population of tumour-infiltrating lymphocytes. The patient's peripheral blood lymphocytes are cultured with interleukin-2 which generates lymphokine-activated killer cells having potent anti-tumour effect.

b) *Cytokine therapy* is used to build up specific and non-specific host defenses. These include: interleukin-2, interferon- α and - γ , tumour necrosis factor- α , and granulocyte-monocyte colony stimulating factor (GM-CSF).

c) *Monoclonal antibody therapy* is currently being tried as tumour cell toxin in the treatment of leukaemias and lymphomas.

DIAGNOSIS OF CANCER

When the diagnosis of cancer is suspected on clinical examination and on other investigations, it must be confirmed. The most certain and reliable method which has stood the test of time is the histological examination of biopsy, though recently many other methods to arrive at the correct diagnosis or confirm the histological diagnosis are available which are discussed in Chapter 2.

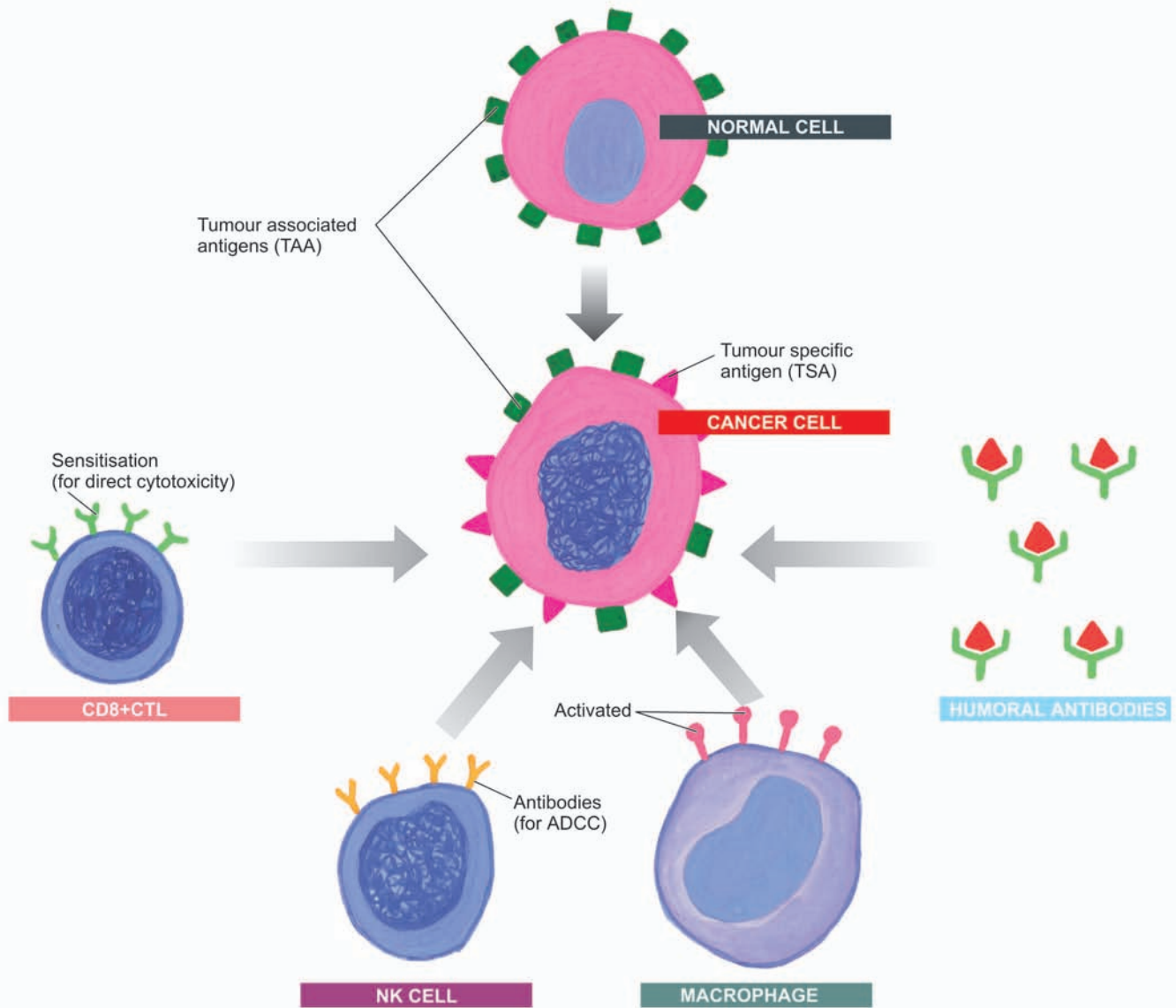
1. Histological Methods

These methods are based on microscopic examination of properly fixed tissue (excised tumour mass or open/needle biopsy from the mass), supported with complete clinical and investigative data. These methods are most valuable in arriving at the accurate diagnosis. The tissue must be fixed in 10% formalin for light microscopic examination and in glutaraldehyde for electron microscopic studies, while quick-frozen section and hormonal analysis are carried out on fresh unfixed tissues.

The histological diagnosis by either of these methods is made on the basis that cytological features of *benign tumours* resemble those of normal tissue and that they are unable to invade and metastasise, while *malignant tumours* are identified by lack of differentiation in cancer cells termed 'anaplasia' or 'cellular atypia' and may invade as well as metastasise. The light microscopic and ultrastructural characteristics of neoplastic cell have already been described in this chapter.

2. Cytological Methods

Cytological methods for diagnosis consist of study of cells shed off into body cavities (exfoliative cytology) and study of cells by putting a fine needle introduced

**FIGURE 22.1**

Schematic illustration of immune responses in cancer. For details see the text (CTL = cytotoxic T-lymphocyte; NK cell = natural killer cell; ADCC = antibody-dependent cellular cytotoxicity).

under vacuum into the lesion (fine needle aspiration cytology, FNAC).

i) Exfoliative cytology. Cytologic smear (Papanicolaou Pap smear) method was initially employed for detecting dysplasia, carcinoma *in situ* and invasive carcinoma of the uterine cervix. However, its use has now been widely extended to include examination of sputum and bronchial washings; pleural, peritoneal and pericardial effusions; urine, gastric secretions, and CSF. The method

is based on microscopic identification of the characteristics of malignant cells which are incohesive and loose and are thus shed off or 'exfoliated' into the lumen. However, a 'negative diagnosis' does not altogether rule out malignancy due to possibility of sampling error.

ii) Fine needle aspiration cytology (FNAC). Currently, cytopathology includes not only study of exfoliated cells but also materials obtained from superficial and deep-seated lesions in the body which do not shed off cells

freely. The latter method consists of study of cells obtained by a fine needle introduced under vacuum into the lesion, so called *fine needle aspiration cytology (FNAC)*. The superficial masses can be aspirated under direct vision while deep-seated masses such as intra-abdominal, pelvic organs and retroperitoneum are frequently investigated by ultrasound (US) or computed tomography (CT) guided fine needle aspirations. The smears are fixed in 95% ethanol by wet fixation or may be air-dried unfixed. While Papanicolaou method of staining is routinely employed in most laboratories for *wet fixed smears*, others prefer H and E due to similarity in staining characteristics in the sections obtained by paraffin embedding. *Air-dried smears* are stained by May-Grunwald-Giemsa or Leishman stain. FNAC has a diagnostic reliability between 80-97% but it must not be substituted for clinical judgement or compete with an indicated histopathologic biopsy.

3. Histochemistry and Cytochemistry

Histochemistry and cytochemistry are additional diagnostic tools which help the pathologist in identifying the chemical composition of cells, their constituents and their products by special staining methods.

Though immunohistochemical techniques are more useful for tumour diagnosis (see below), histochemical and cytochemical methods are still employed for this purpose.

Some of the common examples are summarised in Table 22.2, while the subject is discussed at length on page 10.

4. Immunohistochemistry

This is an immunological method of recognising a cell by one or more of its specific components in the cytoplasm, cell membrane or nucleus. These cell components (called antigens) combine with specific antibodies on the formalin-fixed paraffin sections or cytological smears. The complex of antigen-antibody on slide is made visible for light microscopic identification by either fluorescent dyes ('fluorochromes') or by enzyme system ('chromogens'). The specific antibody against a particular cellular antigen is now-a-days obtained by hybridoma technique for monoclonal antibody production. These monoclonal antibodies, besides being *specific* against antigen, are highly sensitive in detection of antigenic component, and, therefore, impart objectivity to the subjective tumour diagnosis made by the surgical pathologist.

Though the list of immunohistochemical stains is ever increasing, one important group of such antibody

TABLE 22.2: Common Histochemical/Cytochemical Stains in Tumour Diagnosis.

SUBSTANCE	STAIN
1. <i>Basement membrane/collagen</i>	• Periodic acid-Schiff (PAS) • Reticulin • Van Gieson • Masson's trichrome
2. <i>Glycogen</i>	• PAS with diastase loss
3. <i>Glycoproteins, glycolipids (epithelial origin)</i>	• PAS with diastase persistence glycomucins
4. <i>Acid mucin (mesenchymal origin)</i>	• Alcian blue
5. <i>Mucin (in general)</i>	• Combined Alcian blue-PAS
6. <i>Argyrophilic/argentaffin granules</i>	• Silver stains
7. <i>Cross striations</i>	• PTAH stain
8. <i>Enzymes</i>	• Myeloperoxidase • Acid phosphatase • Alkaline phosphatase
9. <i>Nucleolar organiser regions (NORs)</i>	• Colloidal silver stain

stains is directed against various classes of intermediate filaments which is useful in classification of poorly-differentiated tumours of epithelial or mesenchymal origin (Table 22.3). This subject is discussed already on page 13.

5. Electron Microscopy

Ultrastructural examination of tumour cells offers selective role in diagnostic pathology. EM examination may be helpful in confirming or substantiating a tumour diagnosis arrived at by light microscopy and immunohistochemistry. A few general features of malignant tumour cells by EM examination are:

- Cell junctions—their presence and type.
- Cell surface, e.g. presence of microvilli.
- Cell shape and cytoplasmic extensions.
- Shape of the nucleus and features of nuclear membrane.
- Nucleoli—size and density.
- Cytoplasmic organelles—their number is generally reduced.
- Dense bodies in the cytoplasm.
- Any other secretory product in the cytoplasm e.g. melanosomes in melanoma and membrane-bound granules in endocrine tumours.

TABLE 22.3: Intermediate Filaments and their Significance in Tumour Diagnosis.

INTERMEDIATE FILAMENT	TUMOUR
1. <i>Keratins</i>	Carcinomas, mesotheliomas, some germ cells tumours
2. <i>Vimentin</i>	Sarcomas, melanomas, lymphomas
3. <i>Desmin</i>	Myogenic tumours
4. <i>Neurofilaments (NF)</i>	Neural tumours
5. <i>Glial fibrillary acidic protein (GFAP)</i>	Glial tumours

6. Tumour Markers (Biochemical Assays)

In order to distinguish from the preceding techniques of tumour diagnosis in which 'stains' are imparted on the tumour cells in section or smear, tumour markers are biochemical assays of products elaborated by the tumour cells in blood or other body fluids. It is, therefore, pertinent to keep in mind that many of these products are produced by normal body cells too, and thus the biochemical estimation of the product in blood reflects the total substance and not by the tumour cells alone. These methods, therefore, lack sensitivity as well as specificity and can only be employed for:

■ *firstly*, as an adjunct to the pathologic diagnosis arrived at by other methods and not for primary diagnosis of cancer; and

■ *secondly*, can be used for prognostic and therapeutic purposes.

Tumour markers include: cell surface antigens (or oncofoetal antigens), cytoplasmic proteins, enzymes, hormones and cancer antigens (Table 22.4). However, two of the best known examples of oncofoetal antigens secreted by foetal tissues as well as by tumours are alpha-foetoproteins (AFP) and carcinoembryonic antigens (CEA):

a) Alpha-foetoprotein (AFP): This is a glycoprotein synthesised normally by foetal liver cells. Their levels are elevated in hepatocellular carcinoma and non-seminomatous germ cell tumours of the testis. Certain non-neoplastic conditions also have increased levels of AFP e.g. in hepatitis, cirrhosis, toxic liver injury and pregnancy.

b) Carcino-embryonic antigen (CEA): CEA is also a glycoprotein normally synthesised in embryonic tissue of the gut, pancreas and liver. Their levels are high in cancers of the gastrointestinal tract, pancreas and breast. As in AFP, CEA levels are also elevated in certain non-neoplastic conditions e.g. in ulcerative colitis, Crohn's disease, hepatitis and chronic bronchitis.

7. Modern Aids in Tumour Diagnosis

In addition to the methods described above, some more modern diagnostic techniques have emerged for

TABLE 22.4: Important Tumour Markers.

MARKER	CANCER
1. Oncofoetal antigens:	
i. <i>Alpha-foetoprotein (AFP)</i>	Hepatocellular carcinoma, non-seminomatous germ cell tumours of testis
ii. <i>Carcino-embryonic antigen (CEA)</i>	Cancer of bowel, pancreas, breast
2. Cytoplasmic proteins:	
i. <i>Immunoglobulins</i>	Multiple myeloma, other gammopathies
ii. <i>Prostate specific antigen (PSA)</i>	Prostate carcinoma
3. Enzymes:	
i. <i>Prostate acid phosphatase (PAP)</i>	Prostatic carcinoma
ii. <i>Neuron-specific enolase (NSE)</i>	Neuroblastoma, oat cell carcinoma lung
4. Hormones:	
i. <i>Human chorionic gonadotropin (HCG)</i>	Trophoblastic tumours, non-seminomatous germ cell tumours of testis
ii. <i>Calcitonin</i>	Medullary carcinoma thyroid
iii. <i>Catecholamines and vanillyl mandelic acid (VMA)</i>	Neuroblastoma, pheochromocytoma
iv. <i>Ectopic hormone production</i>	Paraneoplastic syndromes
5. Secreted cancer antigens:	
i. <i>CA-125</i>	Ovary
ii. <i>CA-15-3</i>	Breast

pathologic diagnosis but their availability as well as applicability are limited. These methods are discussed in Chapter 2. Briefly, their role in tumour diagnosis is outlined below.

i) Flow cytometry. This is a computerised technique by which the detailed characteristics of individual tumour cells are recognised and quantified and the data can be stored for subsequent comparison too. Since for flow cytometry, single cell suspensions are required to 'flow' through the 'cytometer', it can be employed on blood cells and their precursors in bone marrow aspirates and body fluids, and sometimes on fresh-frozen unfixed tissue. The method employs either identification of cell surface antigen (e.g. in classification of leukaemias and lymphomas), or by the DNA content analysis (e.g. aneuploidy in various cancers).

ii) *In situ* hybridisation. This is a molecular technique by which nucleic acid sequences (cellular/viral DNA and RNA) can be localised by specifically-labelled nucleic acid probe directly in the intact cell (*in situ*) rather than by DNA extraction (see below). *In situ* hybridisation may be used for analysis of certain human tumours by the study of oncogenes aside from its use in diagnosis of viral infection.

iii) Molecular diagnostic techniques. The group of molecular biologic methods in the tumour diagnostic

laboratory are a variety of DNA/RNA-based molecular techniques in which the DNA/RNA are extracted (compared from *in situ* above) from the cell and then analysed. These techniques are highly sensitive, specific and rapid and have revolutionised diagnostic pathology in neoplastic as well as non-neoplastic conditions (e.g. in infectious and inherited disorders, and in identity diagnosis). Molecular diagnostic techniques include: DNA analysis by Southern blot, RNA analysis by northern blot, and polymerase chain reaction (PCR). The molecular methods in tumour diagnosis can be applied in haematologic as well as non-haematologic malignancies by:

- analysis of molecular cytogenetic abnormalities;
- mutational analysis;
- antigen receptor gene rearrangement; and
- by study of oncogenic viruses at molecular level.

iv) DNA microarray analysis of tumours. Currently, it is possible to perform molecular profiling of a tumour by use of gene chip technology which allows measurement of levels of expression of several thousand genes (up-regulation or down-regulation) simultaneously. Fluorescent labels are used to code the cDNA synthesised by trigger from mRNA. The conventional DNA probes are substituted by silicon chip which contains the entire range of genes and high resolution scanners are used for the measurement.



CLASSIFICATION OF TUMOURS

Many types of classification of tumours have been suggested. The most useful and widely accepted classification of tumours is based on the tissue of origin (i.e., histogenesis) and on the anticipated behaviour. In general, on one end are the tumours having expansile growth without stigmata of malignancy; they are essentially *benign tumours*. On the other extreme are tumours having rapidly proliferating growth pattern with local invasion as well as metastatic involvement; they are termed *malignant tumours*. Between the two extremes is, however, another *borderline* or *intermediate group* having localised aggressive growth and invasiveness but seldom having distant metastases e.g. basal cell carcinoma, carcinoid tumour of the appendix, mucoepidermoid tumour of the salivary gland.

As already discussed in chapter 20, the general principle of nomenclature of tumours is to add the suffix *-oma* to the name of tissue of origin. Malignant tumours of epithelial origin are called *carcinomas* while those of mesenchymal origin are termed *sarcomas*.

A classification based on these principles divides all tumours into 2 broad groups: epithelial and mesenchymal (see Table 20.1, page 237). Morphology of some of the common tumours is described below.

EPITHELIAL TUMOURS

Epithelium may be of 2 main types: surface and secretory.

Surface epithelium of squamous type covers the skin, oral cavity, upper part of oesophagus and vagina. The tumours originating from it are called squamous cell papillomas and carcinomas for the benign and malignant tumours respectively. The urinary bladder is lined by transitional epithelium and the tumours in that location are accordingly named transitional cell papilloma and carcinoma.

Secretory epithelium is present in mucous membranes (e.g. in bowel, gallbladder, uterus) and in glandular structures such as in the breast, salivary glands, liver, kidney, prostate, etc. The tumours arising from secretory and glandular epithelium are termed adenomas and adenocarcinomas.

The prototypes of the epithelial group of benign and malignant tumours are discussed below.

BENIGN EPITHELIAL TUMOURS

SQUAMOUS PAPILLOMA. Squamous papilloma is a benign epithelial tumour of the skin. Though considered by many authors to include common viral warts (*verrucae*) and condyloma acuminata, true squamous papillomas differ from these viral lesions. If these 'viral tumours' are excluded, squamous papilloma is a rare tumour.

Histologically, squamous papillomas are characterised by hyperkeratosis, acanthosis with elongation of rete ridges and papillomatosis (Fig. 23.1). The *verrucae*, in addition to these features, have foci of vacuolated cells in the acanthotic stratum malpighii, vertical tiers of parakeratosis between the adjacent papillae and irregular clumps of keratohyaline granules in the valleys between the papillae (**COLOUR PLATE VII: CL 25**).

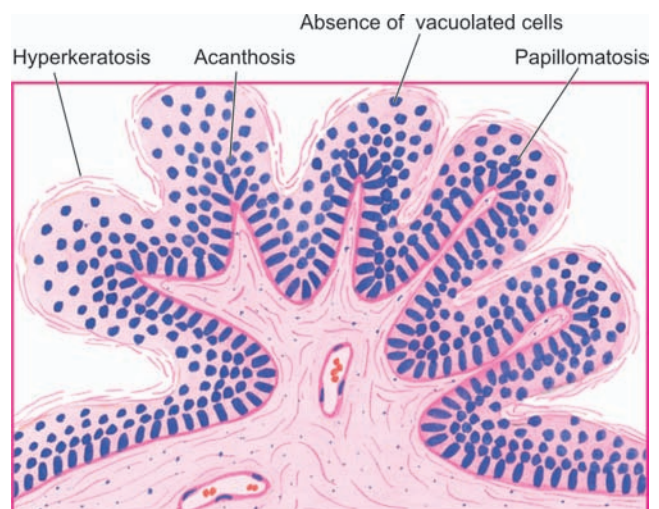


FIGURE 23.1

Squamous cell papilloma. Please note that it differs from verruca by not having vacuolated cells in stratum malpighii.

TRANSITIONAL CELL PAPILLOMA. More than 90% of bladder tumours arise from transitional epithelial (urothelium) lining of the bladder in continuity with the epithelial lining of the renal pelvis, ureters, and the major part of the urethra. Though many workers consider all transitional cell tumours as transitional cell carcinoma, others distinguish true transitional cell papilloma from grade I transitional cell carcinoma. Transitional cell papillomas may occur singly or may be multiple. They are generally small, less than 2 cm in diameter, papillary with branching pattern.

Microscopically, each papilla is composed of fibrovascular stromal core covered by normal looking transitional cells having normal number of layers (not more than six) in thickness. The individual cells resemble the normal transitional cells and do not vary in size and shape. Mitoses are absent and basal polarity is retained. It must be emphasised that the designation transitional cell papilloma is purely a histological diagnosis but does not imply an innocent biologic behaviour. In fact, it may recur and behave in a malignant manner.

ADENOMA. The most common location for adenomas is the bowel, essentially the large intestine. Most of these tumours present as colorectal polyps.

Adenomas have 3 main varieties (tubular, villous and tubulovillous), each of which represents a difference in the growth pattern of the same neoplastic process.

1. Tubular adenoma. Tubular adenoma or adenomatous polyps of the large intestine are the most common neoplastic polyps (75%). They occur most often in the distal colon and rectum. They may be found singly as sporadic cases, or multiple tubular adenomas as part of familial polyposis syndrome with autosomal dominant inheritance pattern. Tubular adenomas may remain asymptomatic or may manifest by rectal bleeding.

Grossly, adenomatous polyps may be single or multiple, sessile or pedunculated, vary in size from less than 1 cm to large, spherical masses with an irregular surface. Usually, the larger lesions have recognisable stalks.

Microscopically, the usual appearance is of benign tumour overlying muscularis mucosa and is composed of branching tubules which are embedded in the lamina propria. The lining epithelial cells are of large intestinal type with diminished mucus-secreting capacity, large nuclei and increased mitotic activity (Fig. 23.2,A).

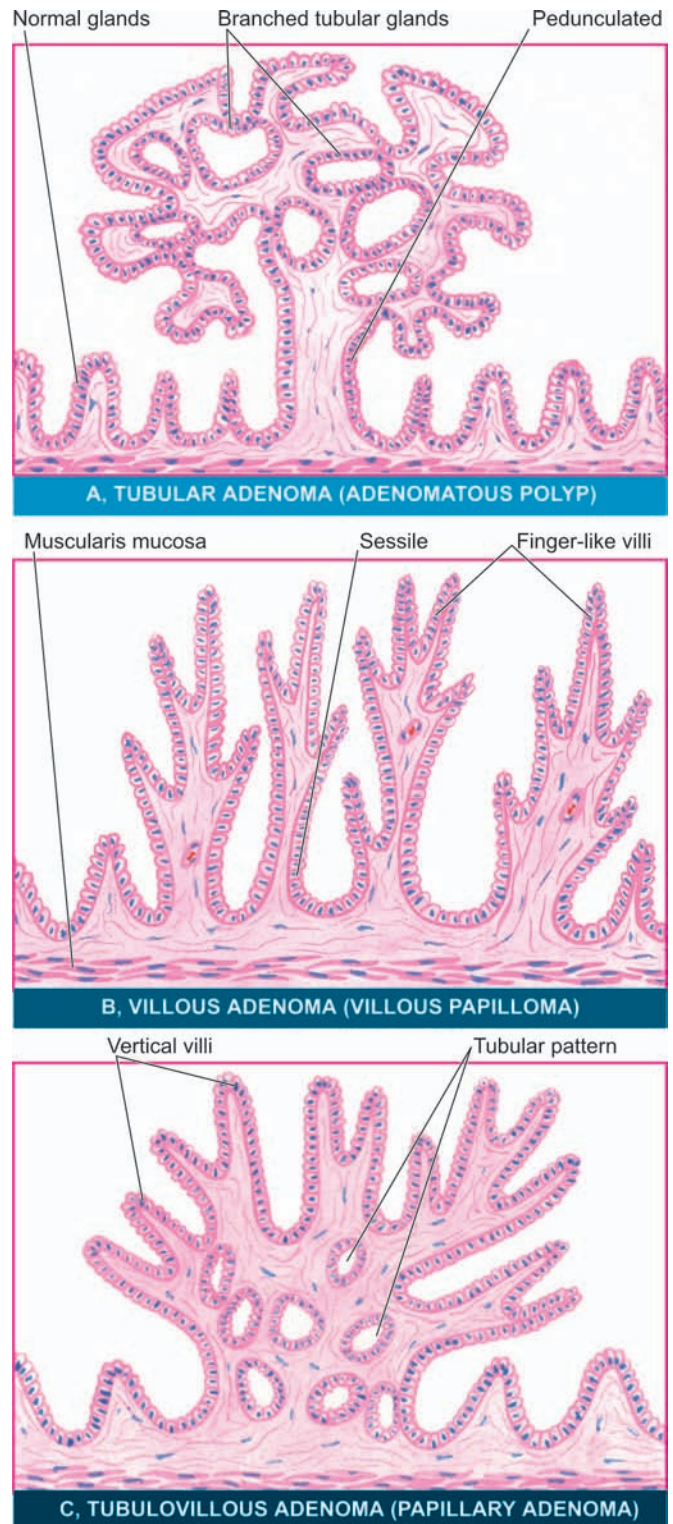


FIGURE 23.2

Adenomas (neoplastic polyps)—three main varieties.

Malignant transformation is present in about 5% of tubular adenomas; the incidence being higher in larger adenomas.

2. Villous adenoma. Villous adenomas or villous papillomas of the colon are much less common than tubular adenomas. The mean age at which they appear is 6th decade of life with approximately equal sex incidence. They are seen most often in the distal colon and rectum, followed in decreasing frequency, by rest of the colon.

Grossly, villous adenomas are round to oval exophytic masses, usually sessile, varying in size from 1 to 10 cm or more in diameter. Their surface may be haemorrhagic or ulcerated. *Microscopically*, the characteristic histologic feature is the presence of many slender, finger-like villi, which appear to arise directly from the area of muscularis mucosae. Each of the papillae have fibrovascular stromal core that is covered by epithelial cells varying from apparently benign to anaplastic cells. Excess mucus secretion is sometimes seen (Fig. 23.2,B).

Villous adenomas are invariably symptomatic; rectal bleeding, diarrhoea and mucus being the common features. The presence of severe atypia, carcinoma *in situ* and invasive carcinoma are seen more frequently. Invasive carcinoma has been reported in 30% of villous adenomas.

3. Tubulovillous adenoma. Tubulovillous adenoma is an intermediate form of pattern between tubular adenoma and villous adenoma. It is also known by other names like papillary adenoma and villo-glandular adenoma. The distribution of these adenomas is the same as for tubular adenomas.

Grossly, tubulovillous adenomas may be sessile or pedunculated and range in size from 0.5-5 cm. *Microscopically*, they show intermediate or mixed pattern, characteristic vertical villi and deeper part showing tubular pattern (Fig. 23.2,C).

The behaviour of tubulovillous adenoma is intermediate between tubular and villous adenomas.

NAEVOCELLULAR NAEVI. Pigmented naevi or moles are extremely common lesions on the skin of most individuals. They are often flat or slightly elevated lesions; rarely they may be papillomatous or pedunculated. Most naevi appear in adolescence and in early adulthood due to hormonal influence but rarely may be present at birth. They are mostly tan to brown and less than 1 cm in size.

Microscopically, irrespective of the histologic types, all naevocellular naevi are composed of 'naevus cells' which are actually identical to melanocytes but differ from melanocytes in being arranged in clusters or nests. Naevus cells are cuboidal or oval in shape with homogeneous cytoplasm and contain large round or oval nucleus. Melanin pigment is abundant in the naevus cells present in the lower epidermis and upper dermis, but the cells in the mid-dermis and lower dermis hardly contain any melanin (Fig. 23.3) (COLOUR PLATE VI: CL 23).

MALIGNANT EPITHELIAL TUMOURS

SQUAMOUS CELL CARCINOMA. Squamous cell carcinoma may arise on any part of the skin and mucous membranes lined by squamous epithelium but is more likely to occur on sun-exposed parts in older people. Various predisposing conditions include: solar keratosis, chronic ulcers, draining sinus, osteomyelitis, old burn scars (Marjolin's ulcers), ionising radiation, industrial carcinogens (coal tars, oils etc), and in the case of cancer of oral cavity, chewing betel nuts and tobacco. Cancer of scrotal skin in chimney-sweeps was the first cancer in which a carcinogen (soot) was implicated. 'Kangri cancer' of the skin of inner side of thigh and lower abdomen, common in natives of Kashmir, is another example of skin cancer due to chronic irritation (Kangri is an earthen pot containing glowing charcoal used by Kashmiris close to their abdomen to keep them warm).

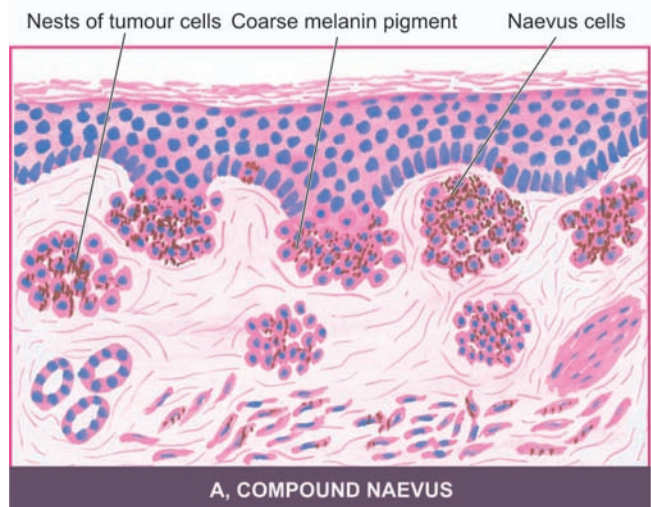


FIGURE 23.3

Compound naevus showing nests of naevus cells which are typically uniform and present in dermis as well as epidermis. Melanin pigment in naevus cells is coarse and irregular.

Although squamous carcinomas can occur anywhere on the skin, most common locations are the face, pinna of the ears, back of hands and mucocutaneous junctions such as on the lips, anal canal and glans penis. Cutaneous squamous carcinoma arising in a pre-existing inflammatory and degenerative lesion has a higher incidence of developing metastases.

Macroscopically, squamous carcinoma of the skin and squamous-lined mucosa can have one of the following two patterns (Fig. 23.4):

- i) More commonly, an ulcerated growth with elevated and indurated margin is seen.
- ii) Less often, a raised fungating or polypoid verrucous lesion without ulceration is found.

Microscopically, squamous cell carcinoma is an invasive carcinoma of the surface epidermis characterised by the following features (Fig. 23.5):

- i) There is irregular downward proliferation of epidermal cells into the dermis.
- ii) Depending upon the grade of malignancy, the masses of epidermal cells show atypical features such as variation in cell size and shape, nuclear hyperchromatism, absence of intercellular bridges, individual cell keratinisation and occurrence of atypical mitotic figures.
- iii) Better-differentiated squamous carcinomas have whorled arrangement of malignant squamous cells forming horn pearls. The centres of these horn pearls may contain laminated keratin material (**COLOUR PLATE VII: CL 26**).
- iv) Higher grades of squamous carcinomas, however, have fewer or no horn pearls and may instead have highly atypical cells.

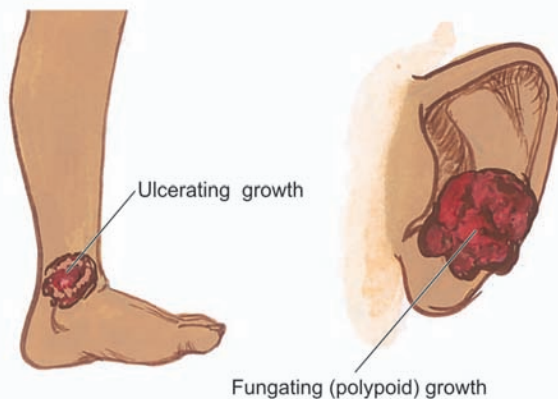


FIGURE 23.4

Macroscopic patterns of squamous cell carcinoma.

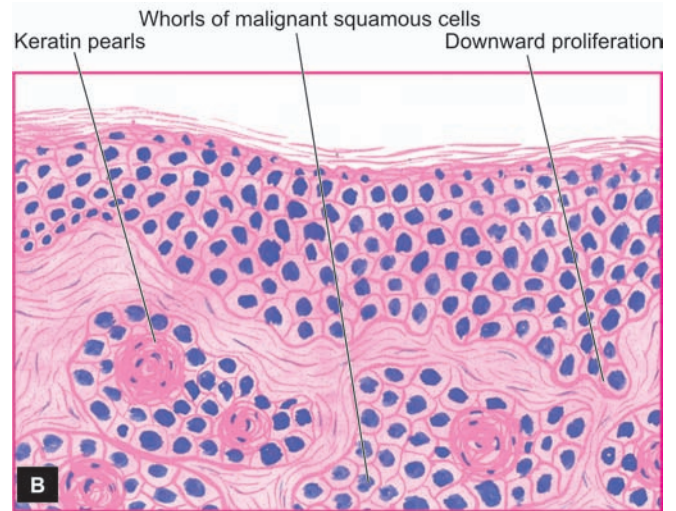


FIGURE 23.5

Squamous cell carcinoma. Microscopic features of well-differentiated squamous cell carcinoma. The dermis is invaded by downward proliferating epidermal masses of cells which show atypical features. A few horn pearls with central laminated keratin are present. There is marked inflammatory reaction in the dermis between the masses of tumour cells.

A system of grading of squamous cell carcinoma called *Broders' grading* has been proposed that depends upon the percentage of anaplastic cells present in a tumour. Accordingly, the following 4 grades are recognised:

Grade I: Less than 25% anaplastic cells

Grade II: 25-50% anaplastic cells

Grade III: 50-75% anaplastic cells

Grade IV: More than 75% anaplastic cells.

However, it is important to take into account other factors for grading the tumour such as: the degree of atypicality of the tumour cells, presence or absence of keratinisation and the depth of penetration of the lesion. Therefore, based on combination of these factors, it is customary with pathologists to label squamous cell carcinomas with descriptive terms such as: well-differentiated, moderately-differentiated, undifferentiated, keratinising, non-keratinising, spindle cell type etc.

BASAL CELL CARCINOMA (RODENT ULCER).

Typically, the basal cell carcinoma is a locally invasive, slow-growing tumour of middle-aged that rarely metastasises. It occurs exclusively on hairy skin, the most common location (90%) being the face, usually above a line from the lobe of the ear to the corner of the mouth (Fig. 23.6). Basal cell carcinoma is seen more frequently in white-skinned people and in those who

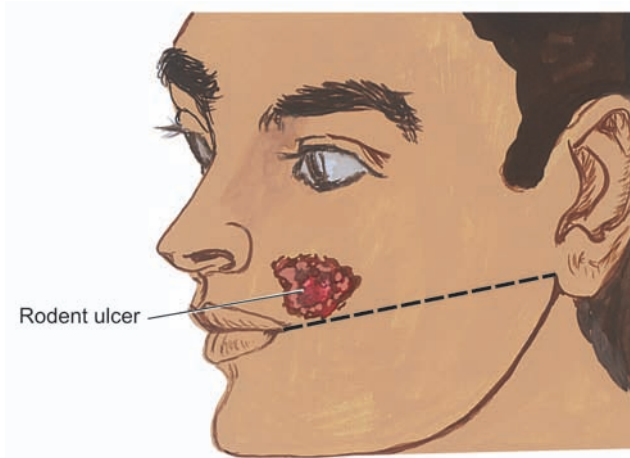


FIGURE 23.6

Common location and macroscopic appearance of basal cell carcinoma (rodent ulcer).

have prolonged exposure to strong sunlight like in those living in Australia.

Macroscopically, the most common pattern is a nodulo-ulcerative basal cell carcinoma in which a slow-growing small nodule undergoes central ulceration with pearly, rolled margins. The tumour enlarges in size by burrowing and by destroying the tissues locally like a rodent and hence the name 'rodent ulcer' (Fig. 23.6). However, less frequently non-ulcerated nodular pattern, pigmented basal cell carcinoma and fibrosing variants are also encountered.

Microscopically, the most characteristic feature is the proliferation of basaloid cells (resembling basal layer of epidermis). A variety of patterns of these cells may be seen: solid masses, masses of pigmented cells, strands and nests of tumour cells in morphea pattern, keratotic masses, cystic change with sebaceous differentiation, and adenoid pattern with apocrine or eccrine differentiation. The most common pattern is *solid basal cell carcinoma* in which the dermis contains irregular masses of basaloid cells having characteristic peripheral palisaded appearance of the nuclei (Fig. 23.7) (COLOUR PLATE VIII: CL 29). A superficial multicentric variant composed of multiple foci of tumour cells present in the dermis, especially on the trunk, has also been described.

MALIGNANT MELANOMA. Malignant melanoma or melanocarcinoma arising from melanocytes is one of

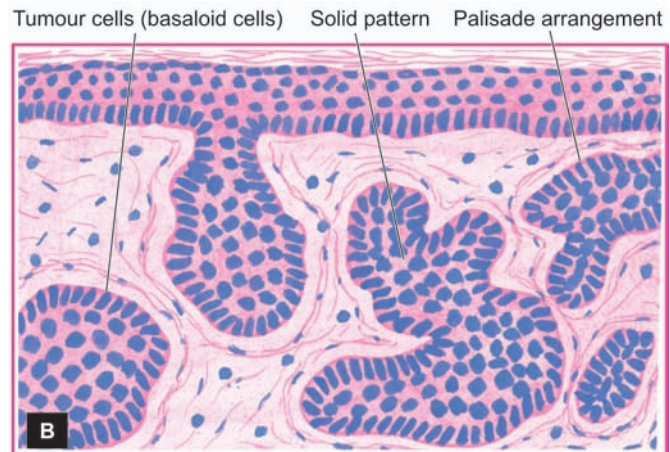


FIGURE 23.7

Solid basal cell carcinoma. The dermis is invaded by irregular masses of basaloid cells with characteristic peripheral palisaded appearance.

the most rapidly spreading malignant tumour of the skin that can occur at all age but is rare before puberty. The tumour spreads locally as well as to distant sites by lymphatics and by blood. The etiology is unknown but there is role of excessive exposure of white skin to sunlight. Other predisposing factors are the presence of pre-existing naevus (especially dysplastic naevus), heredity and local host immune response. Besides the skin, melanomas may occur at various other sites such as oral and anogenital mucosa, oesophagus, conjunctiva, orbit and leptomeninges. The common sites on the skin are the trunk (in men), legs (in women), and face, soles, palms and nail-beds.

Clinically, melanocarcinoma often appears as a flat or slightly elevated naevus which has variegated pigmentation, irregular borders and, of late, has undergone secondary changes or ulceration, bleeding and increase in size. Many of the malignant melanomas, however, arise *de novo* rather than from a pre-existing naevus.

Microscopically, the following characteristics are observed (Fig. 23.8) (COLOUR PLATE VIII: CL 30).

i) **Origin.** The malignant melanoma, whether arising from a pre-existing naevus or starting *de novo*, has marked junctional activity at the epidermo-dermal junction and grows downward into the dermis.

ii) **Tumour cells.** The malignant melanoma cells are usually larger than the naevus cells. They may be epithelioid or spindle-shaped, the former being more common. The tumour cells have amphophilic cyto-

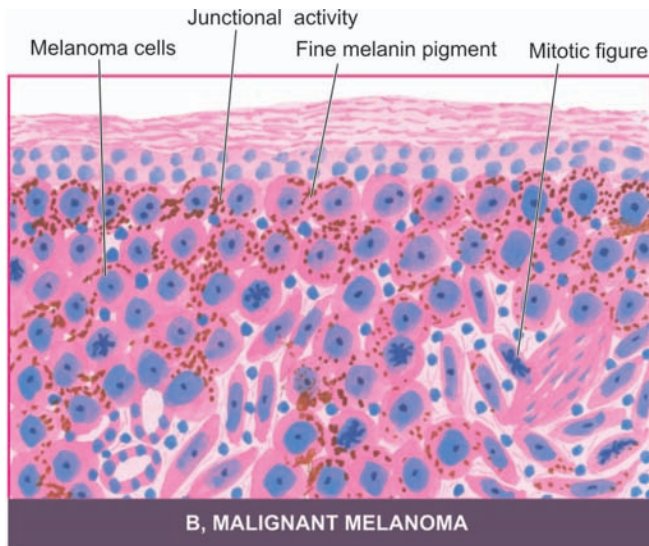


FIGURE 23.8

Malignant melanoma shows junctional activity at the dermal-epidermal junction. Tumour cells resembling epithelioid cells with pleomorphic nuclei and prominent nucleoli are seen as solid masses in the dermis. Many of the tumour cells contain fine granular melanin pigment.

plasm and large, pleomorphic nuclei with conspicuous nucleoli. Mitotic figures are often present and multinucleate giant cells may occur. These tumour cells may be arranged in various patterns such as solid masses, sheets, islands, alveoli etc.

iii) Melanin. Melanin pigment may be present or absent (amelanotic melanoma) without any prognostic influence. The pigment, if present, tends to be in the form of uniform fine granules (unlike the benign naevi in which coarse irregular clumps of melanin are present). At times, there may be no evidence of melanin in H & E stained sections but the Fontanna-Masson stain or *dopa reaction* reveals melanin granules in the cytoplasm of tumour cells.

iv) Inflammatory infiltrate. Some amount of inflammatory infiltrate is present in the invasive melanomas. Infrequently, partial spontaneous regression of the tumour occurs due to destructive effect of dense inflammatory infiltrate.

The prognosis for patients with malignant melanoma is related to the depth of invasion of the tumour in the dermis. Depending upon the depth of invasion below the granular cell layer in millimeters, *Clark* has described 5 levels:

Level I: Malignant melanoma cells confined to the epidermis and its appendages.

Level II: Extension into the papillary dermis.

Level III: Extension of tumour cells up to the interface between papillary and reticular dermis.

Level IV: Invasion of reticular dermis.

Level V: Invasion of the subcutaneous fat.

Metastatic spread of malignant melanoma is very common and takes place via lymphatics to the regional lymph nodes and through blood to distant sites like lungs, liver, brain, spinal cord, and adrenals. Rarely, the primary lesion may regress spontaneously but metastases are present widely distributed.

TRANSITIONAL CELL CARCINOMA. Transitional cell (or urothelial) carcinoma arises from the epithelial lining of the urinary bladder, renal pelvis, ureters and part of urethra. A number of environmental and host factors are implicated in the etiology of the bladder cancer that include: industrial occupations (e.g. in workers engaged in manufacture of aniline dyes, rubber, plastic, textiles and cable), schistosomiasis of the bladder (e.g., in Egypt), tobacco smoking, certain drugs, artificial sweeteners and local lesions such as ectopia vesicae and leukoplakia of the bladder.

As stated before, transitional cell papilloma is distinguished by some workers from grade I transitional cell carcinoma, whereas others do not consider this as a distinct entity.

Grossly, urothelial tumours may be single or multiple. About 90% of the tumours are papillary (non-invasive or invasive), whereas the remaining 10% are flat and indurated (non-invasive or invasive). The papillary tumours have free floating fern-like arrangement with a broad or narrow pedicle. The non-papillary tumours are bulkier with ulcerated surface (Fig. 23.9). More common locations for either of the two types are the trigone, the region of ureteral orifices and on the lateral walls.

Microscopically, transitional cell carcinoma is the commonest cancer of the bladder and is divided into 3 grades depending upon 2 features: the *degree of anaplasia* and the extent of invasion.

The **criteria for anaplasia** are: increased cellularity, nuclear crowding, deranged cellular polarity, failure of normal orientation from base to the surface, variation in cell size and shape, variation in nuclear chromatin pattern, mitotic figures and giant cells.

The **criteria for invasion** in papillary as well as non-papillary tumours are: penetration of the basement membrane of bladder mucosa.

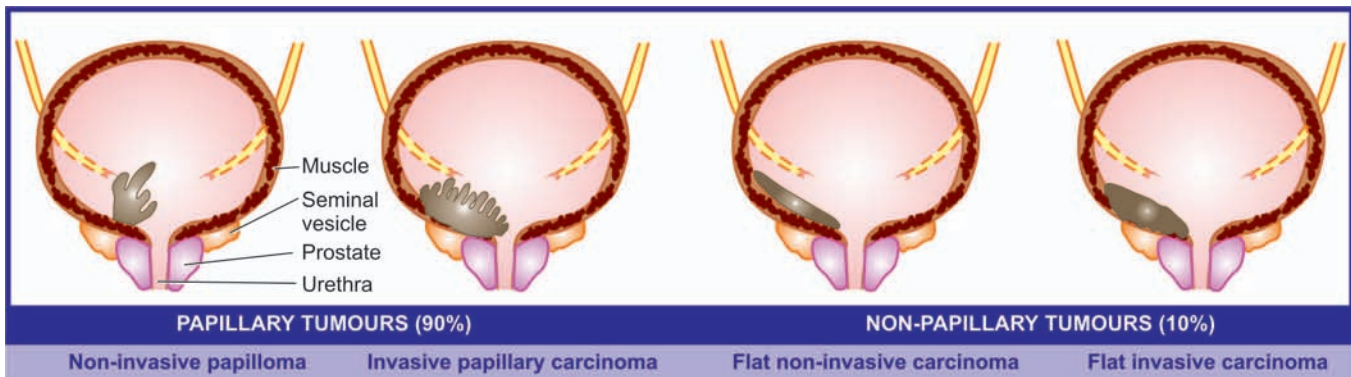


FIGURE 23.9

Macroscopic patterns of epithelial bladder tumours.

Based on these salient features, the characteristics of 3 grades of transitional cell carcinoma are as under:

Grade I: The tumour cells are clearly transitional type but show increased number of layers of cells (c.f. transitional cell papilloma). The individual cells are generally regular but are slightly larger and show mild hyperchromatism.

Grade II: (Fig. 23.10): The tumour cells are still recognisable as of transitional origin and the number of layers of cells is increased. The individual tumour cells are less regular, larger in size, and show pronounced nuclear hyperchromatism, mitotic activity and loss of polarity. The tumour may or may not be invasive.

Grade III: This is the anaplastic or undifferentiated grade of tumour which is always invasive extending into the bladder wall to variable depth depending upon the clinical stage. The tumour cells are no longer recognisable as of transitional origin. The individual tumour cells show pronounced features of anaplasia such as marked pleomorphism, hyperchromatism, total loss of polarity with loosened surface cells exfoliated in the bladder lumen.

There may be foci of squamous or glandular metaplasia in any grade of the tumour.

ADENOCARCINOMA. Carcinoma arising from the secretory epithelium of mucosal surfaces and glandular elements is called adenocarcinoma. The common sites are the stomach, large intestine, gallbladder, pancreas, uterus, prostate, breast and other glandular organs. The spread of tumour can occur both by lymphatic and haematogenous routes.

Grossly, appearance of the tumour is variable depending upon its location. The most common

patterns, however, observed in hollow viscus are either a *fungating (polypoid)* mass protruding into the lumen or an *infiltrating* type invading the wall. In the case of cancer of the stomach, other gross forms seen are scirrhous (linitis plastica), colloid (mucoid), and ulcer-cancer arising in a pre-existing peptic ulcer (Fig. 23.11). In the large intestine, there are distinct differences between the growth on right and left half of the colon: the right-sided growth tending to be large, cauliflower like, soft and friable projecting into the lumen whereas left-sided growths have napkin-ring configuration because the growth encircles the bowel wall circumferentially with increased fibrosis

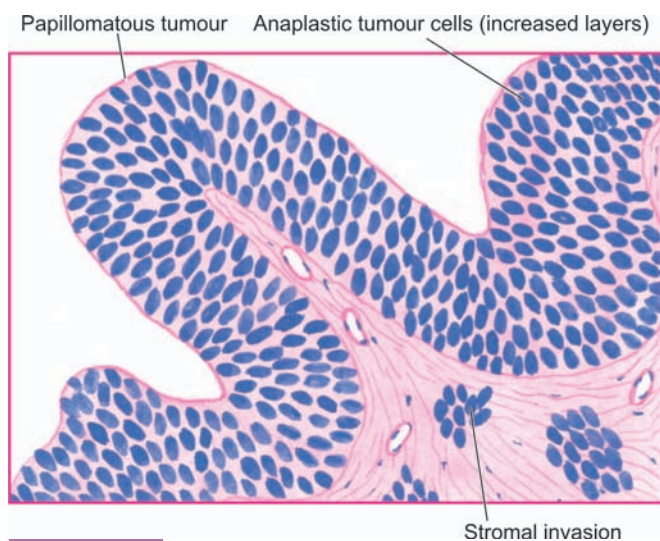


FIGURE 23.10

Microscopic features of grade II transitional cell carcinoma. There is increase in the number of layers of epithelium. The cells are still recognisable as of transitional origin and show features of anaplasia.

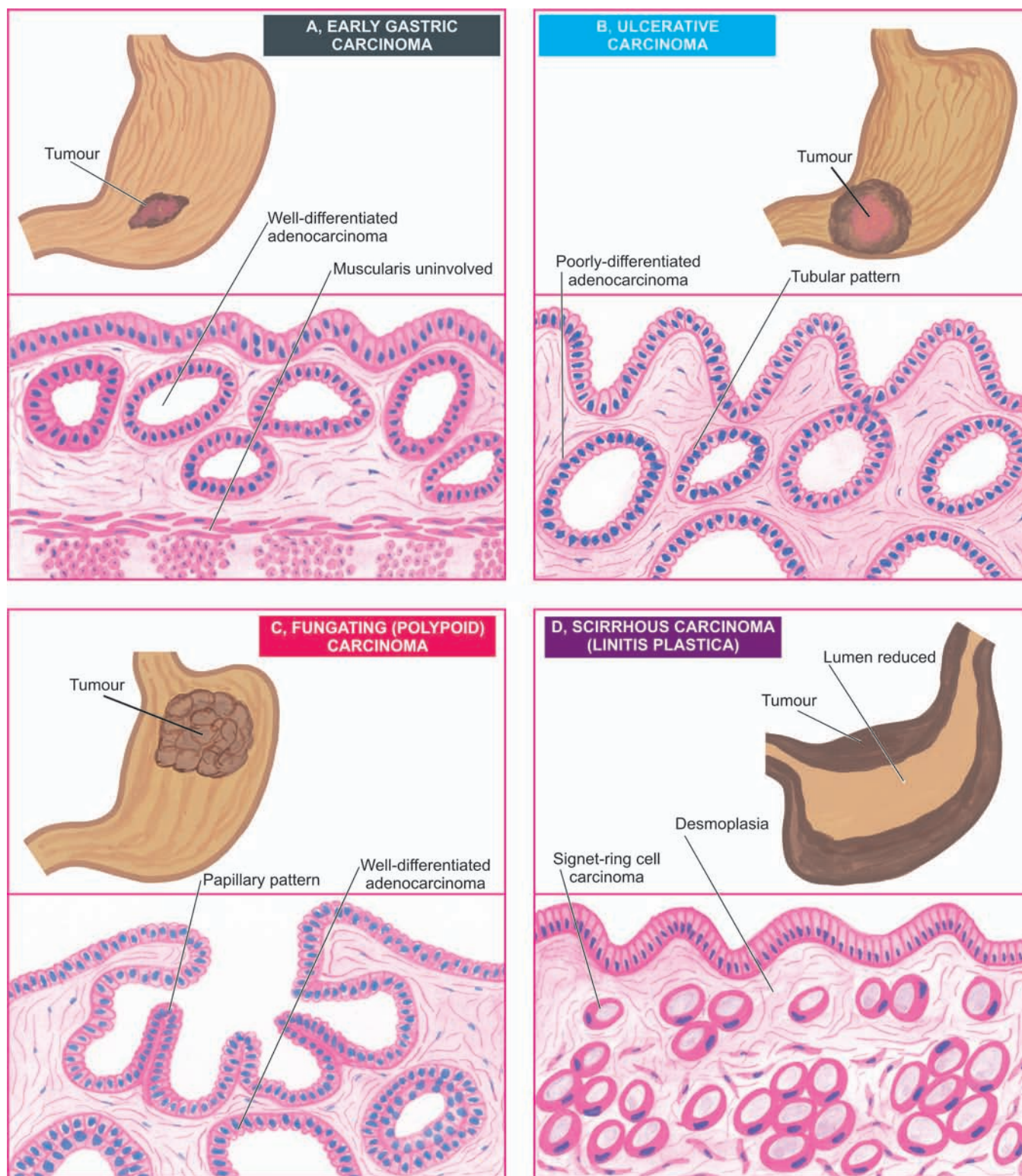


FIGURE 23.11

Adenocarcinoma of the stomach, various macroscopic subtypes (left) and their corresponding dominant histological patterns (right).

forming annular ring. In the breast, the most common gross appearance is of a diffuse infiltrating scirrhous mass with cartilaginous consistency that cuts with a grating sound. The malignant prostate is firm and fibrous and in majority of the cases the cancer is located in the peripheral zone as occult or latent growth.

Microscopically, there is sudden change of the normal mucous membrane to the atypical and irregular malignant epithelium and neoplastic glands invading the layers of the wall of viscus (Fig. 23.11). There may be papillary or infiltrating growth pattern.

The tumour may be mucin-secreting or non-mucin-secreting type. Colloid (mucoid) carcinoma is characterised by large pools of mucin in which are present a small number of tumour cells, sometimes having signet-ring appearance. Scirrhous carcinoma (linitis plastica) of the stomach is an adenocarcinoma in which there is pronounced desmoplasia so much so that cancer cells are difficult to find. Depending upon the degree of differentiation, various histological grades of the adenocarcinoma are described: well-differentiated, moderately-differentiated and poorly-differentiated.

MESENCHYMAL TUMOURS

Mesenchymal or non-epithelial group of tumours includes tumours arising from soft tissues and bones. The soft tissue tumours are considered below while the bone tumours are described in Chapter 33.

For the purpose of classification of this group of tumours, the WHO has defined soft tissues as including all non-epithelial extra-skeletal tissues of the body except the reticuloendothelial system, the glia and the supporting tissues of specific organs and viscera. Thus, tissues included in soft tissues are: fibrous tissue, adipose tissue, muscle tissue, synovial tissue, blood vessels and neuroectodermal tissues of the peripheral and autonomic nervous system. The lesions of these tissues are embryologically derived from mesoderm, except those of peripheral nerve which are derived from ectoderm. Tumours of smooth muscle tissue, blood vessels and nerves are described elsewhere in the book, while tumours and tumour-like lesions composed of other soft tissues are discussed here.

In general, the benign tumours of soft tissues are designated by a prefix indicating the tissue of origin followed by suffix *-oma* and malignant counterparts by adding the suffix *-sarcoma*. Benign soft tissue tumours are about 100 times more common than sarcomas. Sarcomas rarely arise from malignant transformation of a pre-existing benign tumour. Instead, sarcomas

originate from the primitive mesenchymal cells having the capacity to differentiate along different cell pathways. It may be recalled here that soft tissue sarcomas metastasise most frequently by the haematogenous route and disseminate commonly to the lungs, liver, bone and brain. Lymph node metastases are often late and are associated with widespread dissemination of the tumour. Histologic differentiation and grading of soft tissue sarcomas are important because of varying clinical behaviour, prognosis and response to therapy.

A few important principles apply to majority of soft tissue tumours:

- Superficially-located tumours tend to be benign while *deep-seated lesions* are more likely to be malignant.
- *Large-sized tumours* are generally more malignant than small ones.
- A *rapidly-growing tumour* often behaves as a malignant tumour than the one that develops slowly.
- Malignant tumours have frequently *increased vascularity* while benign tumours are selectively avascular.
- Although soft tissue tumours may arise anywhere in the body but in general *more common locations* are: lower extremity (40%), upper extremity (20%), trunk and retroperitoneum (30%) and head and neck (10%).
- In general *males* are affected more commonly than females.
- Approximately 15% of soft tissue tumours occur in *children* and includes some specific examples of soft tissue sarcomas e.g. rhabdomyosarcoma, synovial sarcoma.

ETIOLOGY AND PATHOGENESIS. Etiology of soft tissue tumours remains largely unknown; however a few common features characterise many soft tissue tumours:

1. Frequently there is history of antecedent *trauma* which may bring the tumour to attention of the patient.
2. Molecular and cytogenetic studies in many soft tissue tumours reveal *chromosomal abnormalities and mutations* in genes which can be used as a marker for diagnosis and histogenesis e.g. translocations, various fusion genes etc.
3. Most of the soft tissue tumours occur *sporadically*; however there are a few examples which are components of *genetic syndromes* e.g. neurofibromatosis type 1, Li-Fraumeni syndrome, Osler-Weber-Rendu syndrome etc.

CLASSIFICATION AND DIAGNOSTIC CRITERIA.

Soft tissue tumours are generally classified clinically on the basis of tissue produced by the tumour e.g. fibrous, lipogenic etc. However, accurate pathological diagnosis of soft tissue tumours is based on histogenesis which is

important for determining the prognosis and can be made by the following plan:

1. Cell patterns: Several morphological patterns in which tumour cells are arranged are peculiar in different tumours e.g.

i) *Smooth muscle tumours:* interlacing fascicles of pink staining tumour cells.

ii) *Fibrohistiocytic tumours:* characteristically have storiform pattern in which spindle tumour cells radiate from the centre in a spokedwheel manner.

iii) *Herringbone pattern:* is seen in fibrosarcoma in which the tumour cells are arranged like the vertebral column of seafish.

iv) *Pallisaded arrangement:* characteristically is seen in schwannomas in which the nuclei of tumour cells are piled upon each other.

v) *Biphasic pattern:* is the term used for a combination arrangement of two types—fascicles and epithelial-like; e.g. in synovial sarcoma.

2. Cell types: After looking at the pattern of cells described above, preliminary categorisation of soft tissue tumours is done on the basis of cell types comprising the soft tissue tumour:

i) *Spindle cells:* These are the most common cell types in most sarcomas. However, there are subtle differences in different types of spindle cells e.g.

a) *fibrogenic tumours* have spindle cells with light pink cytoplasm and tapering-ended nuclei;

b) *neurogenic (schwann cell) tumours* have tumour cells similar to fibrogenic cells but have curved nuclei;

c) *leiomyomatous tumours* have spindle cells with blunt-ended (cigar-shaped) nuclei and more intense eosinophilic cytoplasm; and

d) *skeletal muscle tumours* have spindle cells similar to leiomyomatous cells but in addition have cytoplasmic striations.

ii) *Small round cells:* Some soft tissue sarcomas are characterised by dominant presence of small round cells or blue cells and are accordingly termed as malignant small round cell tumours, round cell sarcomas, or blue cell tumours (due to presence of lymphocyte-like nuclear size and dense blue chromatin). For example:

a) rhabdomyosarcoma (embryonal and alveolar types);

b) primitive neuroectodermal tumour (PNET),

c) Ewing's sarcoma;

d) neuroblastoma; and

e) malignant lymphomas.

A few examples of epithelial tumours such as small cell carcinoma and malignant carcinoid tumours are also included in the differential diagnosis of small round cell tumours.

iii) *Epithelioid cells:* Some soft tissue tumours have either epithelioid cells as the main cells (e.g. epithelioid

sarcoma) or have epithelial-like cells as a part of biphasic pattern of the tumour (e.g. synovial sarcoma).

3. Immunohistochemistry: Soft tissue tumours are distinguished by application of immunohistochemical stains. Antibody stains are available against almost each cell constituent. Based on differential diagnosis made on routine morphology, the panel of antibody stains is chosen for applying on paraffin sections for staining. Some common examples are:

i) smooth muscle actin (SMA) for smooth muscle tumours;

ii) vimentin as common marker to distinguish mesenchymal cells from epithelium;

iii) desmin for skeletal muscle cells;

iv) S-100 for nerve fibres;

v) factor VIII antigen for vascular endothelium; and

vi) LCA (leucocyte common antigen) common marker for lymphocytes.

4. Electron microscopy: EM as such is mainly a research tool and does not have much diagnostic value in soft tissue tumours but can be applied sometimes to look for tonofilaments or cell organelles.

5. Cytogenetics: As mentioned above, many soft tissue tumours have specific genetic and chromosomal changes which can be done for determining histogenesis, or for diagnosis and prognosis.

After these brief general comments, a few important examples of tumours of different types of mesenchymal tissue origin are described below.

TUMOURS AND TUMOUR-LIKE LESIONS OF FIBROUS TISSUE

Fibromas, fibromatosis and fibrosarcoma are benign, tumour-like, and malignant neoplasms respectively, of fibrous connective tissue.

FIBROMAS. True fibromas are uncommon tumours in soft tissues. Many fibromas are actually examples of hyperplastic fibrous tissue rather than true neoplasms. On the other hand, combinations of fibrous growth with other mesenchymal tissue elements are more frequent e.g. neurofibroma, fibromyoma, etc.

Three types of fibromas are distinguished:

1. Fibroma durum is a benign, often pedunculated and well-circumscribed tumour occurring on the body surfaces and mucous membranes. It is composed of fully matured and richly collagenous fibrous connective tissue.

2. Fibroma molle or fibrolipoma, also termed **soft fibroma**, is similar type of benign growth composed of mixture of mature fibrous connective tissue and adult-type fat.

3. Elastofibroma is a rare benign fibrous tumour located in the sub-scapular region. It is characterised by association of collagen bundles and branching elastic fibres.

FIBROMATOSIS. 'Fibromatosis' is the term used for tumour-like lesions of fibrous tissue which continue to proliferate actively and may be difficult to differentiate from sarcomas. These lesions may, therefore, be regarded as non-metastasising fibroblastic tumours which tend to invade locally and recur after surgical excision. In addition, electron microscopy has shown that the cells comprising these lesions have features not only of fibroblasts, but of both fibroblasts and smooth muscle cells, the so called *myofibroblasts*.

Depending upon the anatomic locations and the age group affected, fibromatoses are broadly grouped as under:

A. Infantile or juvenile fibromatoses include: fibrous hamartoma of infancy, fibromatosis colli, diffuse infantile fibromatosis, juvenile aponeurotic fibroma, juvenile nasopharyngeal angiofibroma and congenital (generalised and solitary) fibromatosis.

B. Adult type of fibromatoses are: palmar and plantar fibromatosis, nodular fasciitis, cicatricial fibromatosis, keloid, irradiation fibromatosis, penile fibromatosis (Peyronie's disease), abdominal and extra-abdominal desmoid fibromatosis and retroperitoneal fibromatosis.

FIBROSARCOMA. The number of soft tissue tumours being diagnosed as fibrosarcoma has dropped, partly

because of re-classification of fibromatoses which have aggressive and recurrent behaviour, and partly due to inclusion of many of such tumours in the group of fibrous histiocytomas.

Fibrosarcoma is a slow-growing tumour, affecting adults between 4th and 7th decades of life. Most common locations are the lower extremity (especially thigh and around the knee), upper extremity, trunk, head and neck, and retroperitoneum. The tumour is capable of metastasis, chiefly via the bloodstream.

Grossly, fibrosarcoma is a grey-white, firm, lobulated and characteristically circumscribed mass. Cut surface of the tumour is soft, fish-flesh-like, with foci of necrosis and haemorrhages.

Microscopically, the tumour is composed of uniform, spindle-shaped fibroblasts arranged in intersecting fascicles. In well-differentiated tumours, such areas produce '*herring-bone pattern*' (herring is a sea fish) (Fig. 23.12). Poorly-differentiated fibrosarcomas, however, have highly pleomorphic appearance with frequent mitoses and bizarre cells.

FIBROHISTIOCYTIC TUMOURS

Fibrohistiocytic group of tumours is the latest addition to the list of soft tissue tumours. The group as a whole is characterised by distinctive light microscopic features that include presence of cells with fibroblastic and histiocytic features in varying proportions and identifi-

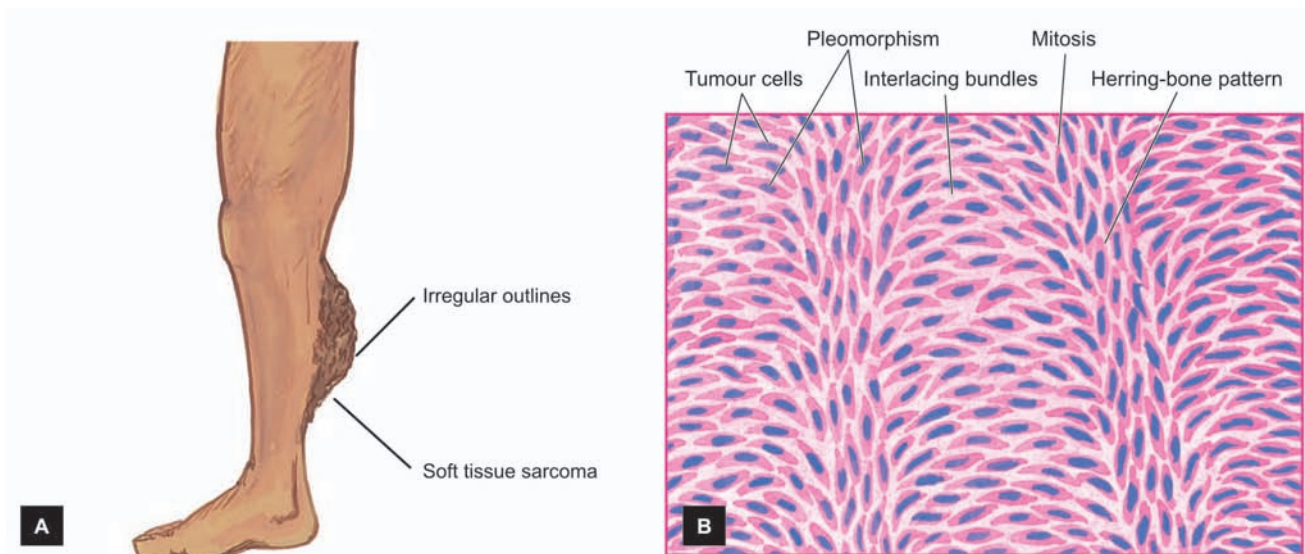


FIGURE 23.12

Fibrosarcoma. A, Common clinical location. B, Microscopy shows a well-differentiated tumour composed of spindle-shaped cells forming interlacing fascicles producing a typical herring-bone pattern.

cation of characteristic *cart-wheel* or *storiform pattern* in which the spindle cells radiate outward from the central focus. The histogenesis of these cells is uncertain but possibly they arise from primitive mesenchymal cells which are capable of differentiating along different cell lines. The group includes full spectrum of lesions varying from *benign* (benign fibrous histiocytoma) to *malignant* (malignant fibrous histiocytoma), with dermatofibrosarcoma protuberans occupying the *intermediate* (low-grade malignancy) position.

BENIGN FIBROUS HISTIOCYTOMAS. Depending upon the location and predominant pattern, benign fibrous histiocytomas include a number of diverse entities such as dermatofibroma, sclerosing haemangioma, fibroxanthoma, xanthogranuloma, giant cell tumour of tendon sheath and pigmented villonodular synovitis. All these tumours have mixed composition of benign fibroblastic and histiocytic pattern of cells.

DERMATOFIBROSARCOMA PROTUBERANS. Dermatofibrosarcoma protuberans is a low-grade malignant cutaneous tumour of disputed fibrohistiocytic origin. The tumour recurs locally, and in rare instances gives rise to distant metastases. Most frequent location is the trunk.

Grossly, the tumour forms a firm, solitary or multiple, satellite nodules extending into the subcutaneous fat and having thin and ulcerated skin surface.

Microscopically, the tumour is highly cellular and is composed of fibroblasts arranged in a cart-wheel or storiform pattern.

MALIGNANT FIBROUS HISTIOCYTOMA. Malignant fibrous histiocytomas, first described about 20 years back, represent approximately 20-30% of all soft tissue sarcomas. The tumour occurs more commonly in males and more frequently in the age group of 5th to 7th decades. Most common locations are lower and upper extremities and retroperitoneum. It begins as a painless, enlarging mass, generally in relation to skeletal muscle, deep fascia or subcutaneous tissue. The histogenesis continues to be elusive but is believed to arise from primitive mesenchymal cells which are capable of differentiating towards both fibroblastic and histiocytic cell line.

Grossly, malignant fibrous histiocytoma is a multilobulated, well-circumscribed, firm or fleshy mass, 5-10 cm in diameter. Cut surface is grey-white, soft and myxoid.

Microscopically, there is marked variation in appearance from area to area within the same tumour. In

general, there is admixture of spindle-shaped *fibroblast-like cells* and mononuclear round to oval *histiocyte-like cells* which may show phagocytic function. There is tendency for the spindle-shaped cell to be arranged in characteristic cart-wheel or storiform pattern. The tumour cells show varying degree of pleomorphism, hyperchromatism, mitotic activity and presence of multinucleate bizarre tumour giant cells. Usually, there are numerous blood vessels and some scattered lymphocytes and plasma cells (Fig. 23.13). The myxoid variant shows areas of loose myxoid stroma in the cellular areas.

Prognosis is determined by the location of the tumour. Deep-seated malignant fibrous histiocytomas such as of the retroperitoneum have poorer prognosis than those of the subcutaneous soft tissue which come to attention earlier. Metastases are frequent, most often to the lungs and regional lymph nodes. Five-year survival rate is approximately 30-50%.

TUMOURS OF ADIPOSE TISSUE

Lipomas and liposarcomas are the common examples of benign and malignant tumours respectively of adipose tissue.

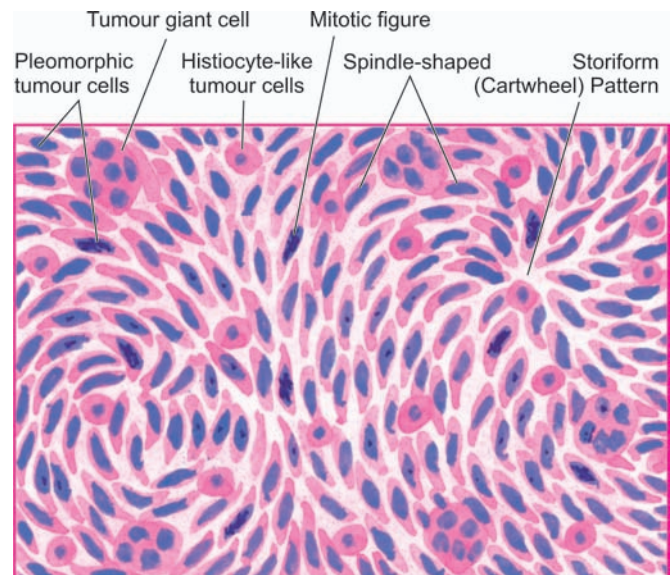


FIGURE 23.13

Malignant fibrous histiocytoma. The tumour shows admixture of spindle-shaped pleomorphic cells forming storiform (cart-wheel) pattern and histiocyte-like round to oval cells. Bizarre pleomorphic multinucleate tumour giant cells and some mononuclear inflammatory cells are also present.

LIPOMA. Lipoma is the commonest soft tissue tumour. It appears as a solitary, soft, movable and painless mass which may remain stationary or grow slowly. Lipomas occur most often in 4th to 5th decades of life and are frequent in females. They may be found at different locations in the body but most common sites are the subcutaneous tissues in the neck, back and shoulder. A lipoma rarely ever transforms into liposarcoma.

Grossly, a subcutaneous lipoma is usually small, round to oval and encapsulated mass. The cut surface is soft, lobulated, yellowish-orange and greasy.

Microscopically, the tumour is composed of lobules of mature adipose cells separated by delicate fibrous septa. A thin fibrous capsule surrounds the tumour (Fig. 23.14) (COLOUR PLATE VII: CL 27).

LIPOSARCOMA. Liposarcoma is one of the most common soft tissue sarcoma, perhaps next in frequency only to malignant fibrous histiocytoma. Unlike lipoma which originates from mature adipose cells, liposarcoma arises from primitive mesenchymal cells, the *lipoblasts*. The peak incidence is in 5th to 7th decades of life. In contrast to lipomas which are more frequently subcutaneous in location, liposarcomas often occur in the deep tissues. Most frequent sites are intermuscular regions in the thigh, buttocks and retroperitoneum.

Grossly, liposarcoma appears as a nodular mass, 5 cm or more in diameter. The tumour is generally

circumscribed but infiltrative. Cut surface is grey-white to yellow, myxoid and gelatinous. Retroperitoneal masses are generally much larger.

Microscopically, the hallmark of diagnosis of liposarcoma is the identification of variable number of *lipoblasts* which may be univacuolated or multivacuolated (Fig. 23.15). The vacuoles represent fat in the cytoplasm. Four major histologic varieties of liposarcomas are distinguished: well-differentiated, myxoid, round cell, and pleomorphic (COLOUR PLATE VII: CL 28).

1. *Well-differentiated liposarcoma* resembles lipoma but contains uni- or multi-vacuolated lipoblasts.
2. *Myxoid liposarcoma* is the most common histologic type. It is composed of monomorphic, fusiform or stellate cells representing primitive mesenchymal cells, lying dispersed in mucopolysaccharide-rich ground substance. Occasional tumour giant cells may be present. Prominent meshwork of capillaries forming chicken-wire pattern is a conspicuous feature.
3. *Round cell liposarcoma* is composed of uniform, round to oval cells having fine multivacuolated cytoplasm with central hyperchromatic nuclei. Round cell liposarcoma may resemble a signet-ring carcinoma but mucin stains help in distinguishing the two.
4. *Pleomorphic liposarcoma* is highly undifferentiated and the most anaplastic type. There are numerous large tumour giant cells and bizarre lipoblasts.

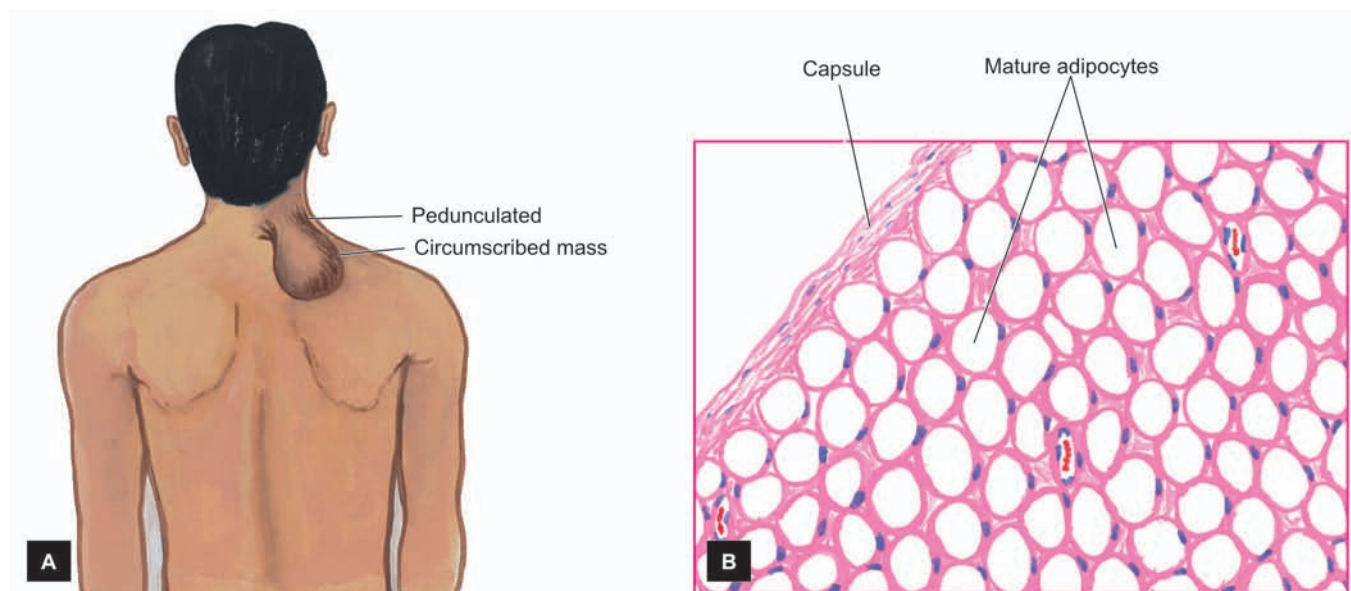


FIGURE 23.14

Lipoma. A, Common clinical location. B, Microscopy of the tumour shows a thin capsule and underlying lobules of mature adipose cells separated by delicate fibrous septa.

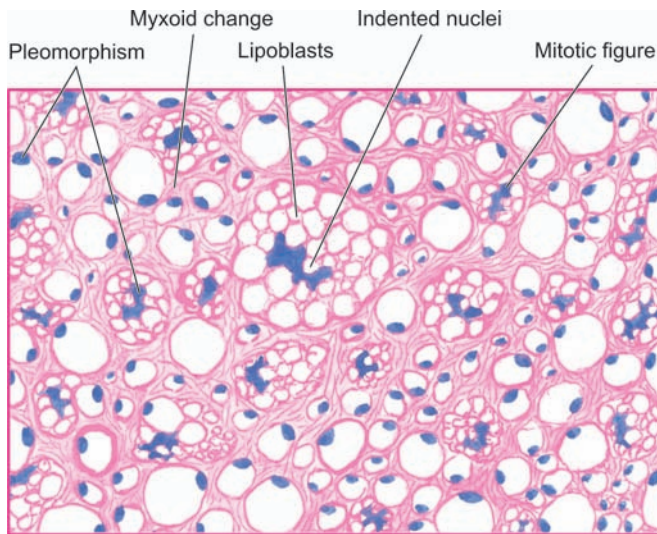


FIGURE 23.15

Liposarcoma, showing characteristic, univacuolated and multivacuolated lipoblasts with bizarre nuclei.

The prognosis of liposarcoma depends upon the location and histologic type. In general, well differentiated and myxoid varieties have excellent prognosis, while pleomorphic liposarcoma has significantly poor prognosis. Round cell and pleomorphic variants metastasise frequently to the lungs, other visceral organs and serosal surfaces.

SMOOTH MUSCLE TUMOURS

Benign and malignant tumours arising from the smooth muscle such as in the uterus, bowel, blood vessel wall etc are called leiomyoma and leiomyosarcoma respectively (*leios* = smooth).

LEIOMYOMA. Leiomyomas or fibromyomas, commonly called *fibroids* by the gynaecologists, are the most common uterine tumours of smooth muscle origin, often admixed with variable amount of fibrous tissue component. About 20% of women above the age of 30 years harbour uterine myomas of varying size. Vast majority of them are benign and cause no symptoms. Malignant transformation occurs in less than 0.5% of leiomyomas. Symptomatic cases may produce abnormal uterine bleeding, pain, symptoms due to compression of surrounding structures and infertility.

The cause of leiomyomas is unknown but the possible stimulus to their proliferation is oestrogen. This is evidenced by increase in their size in pregnancy and high dose oestrogen-therapy and their regression following menopause and castration. Other possible factors implicated in its etiology are human growth hormone and sterility.

Leiomyomas are most frequently located in the uterus where they may occur within the myometrium (*intramural or interstitial*), the serosa (subserosal) or just underneath the endometrium (submucosal). Subserosal and submucosal leiomyomas may develop pedicles and protrude as pedunculated myomas. Leiomyomas may involve the cervix or broad ligament.

Grossly, irrespective of their location, leiomyomas are often multiple, circumscribed, firm, nodular, grey-white masses of variable size. On cut section, they exhibit characteristic whorled pattern (Fig. 23.16,A).

Microscopically, they are essentially composed of 2 tissue elements—whorled bundles of smooth muscle cells admixed with variable amount of connective tissue. The smooth muscle cells are uniform in size and shape with abundant cytoplasm and central oval nuclei (Fig. 23.16, B). *Cellular leiomyomas* have preponderance of smooth muscle elements and may superficially resemble leiomyosarcomas but are distinguished from them by the absence of mitoses.

The pathologic appearance may be altered by secondary changes in the leiomyomas. These include: hyaline degeneration, cystic degeneration, calcification, infection and suppuration, necrosis, fatty change, and rarely, sarcomatous change.

LEIOMYOSARCOMA. Leiomyosarcoma is an uncommon malignant tumour as compared to its rather common benign counterpart. The incidence of malignancy in pre-existing leiomyoma is less than 0.5% but primary uterine sarcoma is less common than that which arises in the leiomyoma. The peak age incidence is seen in 4th to 6th decades of life. The symptoms produced are non-specific such as uterine enlargement and abnormal uterine bleeding.

Grossly, the tumour may form a diffuse, bulky, soft and fleshy mass, or a polypoid mass projecting into lumen.

Microscopically, though there are usually some areas showing whorled arrangement of spindle-shaped smooth muscle cells having large and hyperchromatic nuclei, the hallmark of diagnosis and prognosis is the number of mitoses per high power field (HPF). The essential diagnostic criteria are: more than 10 mitoses per 10 HPF with or without cellular atypia. or 5-10 mitoses per 10 HPF with cellular atypia. More the number of mitoses per 10 HPF, worse is the prognosis.

Leiomyosarcoma is liable to recurrence after removal and eventually metastasises to distant sites such as lungs, liver, bone and brain.

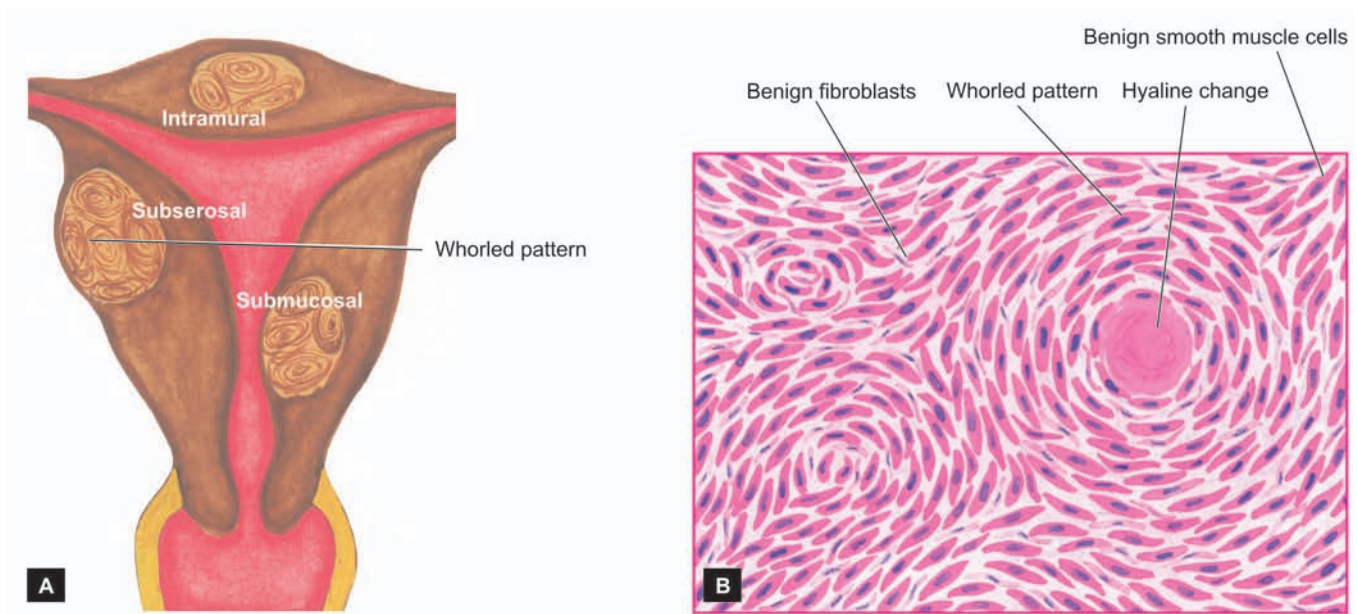


FIGURE 23.16

Leiomyomas. A, Common locations. Cut surface shows characteristic whorled appearance. B, Microscopy shows whorls of smooth muscle cells which are spindle-shaped, having abundant cytoplasm and oval nuclei.

SKELETAL MUSCLE TUMOURS

Rhabdomyoma and rhabdomyosarcoma are the benign and malignant tumours respectively of striated muscle.

RHABDOMYOMA. Rhabdomyoma is a rare benign soft tissue tumour. The common locations are in the head and neck, most often in the upper neck, tongue, larynx and pharynx.

Microscopically, the tumour is composed of large, round to oval cells, having abundant, granular, eosinophilic cytoplasm which is frequently vacuolated and contains glycogen. Cross-striations are generally demonstrable in some cells with phosphotungstic acid-haematoxylin (PTAH) stain. The tumour is divided into adult and foetal type, depending upon the degree of resemblance of tumour cells to normal muscle cells.

RHABDOMYOSARCOMA. Rhabdomyosarcoma is a much more common soft tissue tumour than rhabdomyoma, and is the commonest soft tissue sarcoma in children and young adults. It is a highly malignant tumour arising from rhabdomyoblasts in varying stages of differentiation with or without demonstrable cross-striations. Depending upon the growth pattern and histology, 4 types are distinguished: embryonal, botryoid, alveolar and pleomorphic.

1. Embryonal rhabdomyosarcoma. The embryonal form is the most common of the rhabdomyosarcomas. It occurs predominantly in children under 12 years of age. The common locations are in the head and neck region, most frequently in the orbit, urogenital tract and the retroperitoneum.

Grossly, the tumour forms a gelatinous mass growing between muscles or in the deep subcutaneous tissues but generally has no direct relationship to the skeletal muscle.

Microscopically, the tumour cells have resemblance to embryonal stage of development of muscle fibres. There is considerable variation in cell types. Generally, the tumour consists of a mixture of small, round to oval cells and spindle-shaped strap cell having tapering bipolar cytoplasmic processes in which cross-striations may be evident. The tumour cells form broad fascicles or bands. Mitoses are frequent.

2. Botryoid rhabdomyosarcoma. Botryoid variety is regarded as a variant of embryonal rhabdomyosarcoma occurring in children under 10 years of age. It is seen most frequently in the vagina, urinary bladder and nose.

Grossly, the tumour forms a distinctive grape-like gelatinous mass protruding into the hollow cavity.

Microscopically, the tumour grows underneath the mucosal layer, forming the characteristic *cambium layer* of tumour cells. The tumour is hypocellular and myxoid with predominance of small, round to oval tumour cells.

3. Alveolar rhabdomyosarcoma. Alveolar type of rhabdomyosarcoma is more common in older children and young adults under the age of 20 years. The most common locations, unlike the embryonal variety, are the extremities.

Grossly, the tumour differs from embryonal type in arising directly from skeletal muscle and grows rapidly as soft and gelatinous mass.

Microscopically, the tumour shows characteristic alveolar pattern resembling pulmonary alveolar spaces. These spaces are formed by fine fibrocollagenous septa. The tumour cells lying in these spaces and lining the fibrous trabeculae are generally small, lymphocyte-like with frequent mitoses and some multinucleate tumour giant cells (Fig. 23.17). Cross-striations can be demonstrated in about a quarter of cases.

4. Pleomorphic rhabdomyosarcoma. This less frequent variety of rhabdomyosarcoma occurs predominantly in older adults above the age of 40 years. They are most

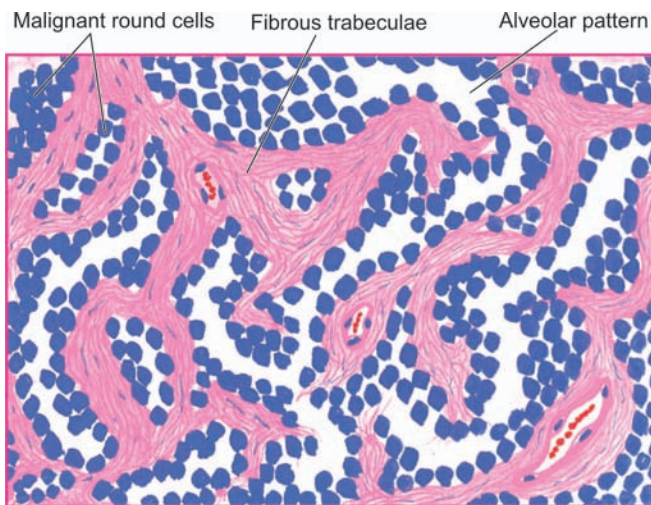


FIGURE 23.17

Alveolar rhabdomyosarcoma. The tumour is divided into alveolar spaces composed of fibrocollagenous tissue. The fibrous trabeculae are lined by small, dark, undifferentiated tumour cells, with some cells floating in the alveolar spaces. A few multinucleate tumour giant cells are also present.

common in the extremities, most frequently in the lower limbs.

Grossly, the tumour forms a well-circumscribed, soft, whitish mass with areas of haemorrhages and necrosis.

Microscopically, the tumour cells show considerable variation in size and shape. The tumour is generally composed of highly anaplastic cells having bizarre appearance and numerous multinucleate giant cells. Various shapes include racquet shape, tadpole appearance, large strap cells, and ribbon shapes containing several nuclei in a row. Cross-striations can be demonstrated with PTAH stain in a few tumours.

VASCULAR TUMOURS

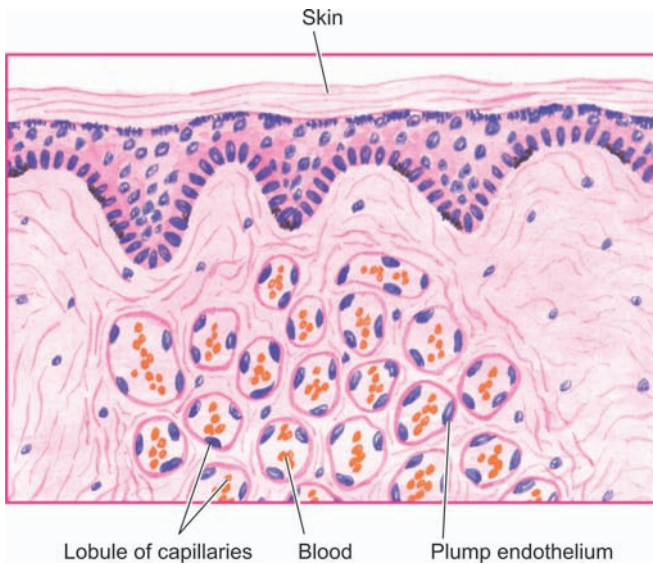
Majority of benign vascular tumours are malformations or hamartomas. A hamartoma is a tumour-like lesion made up of tissues indigenous to the part but lacks the true growth potential of true neoplasms.

However, there is no clear-cut distinction between vascular hamartomas and true benign tumours and are often described together. On the other hand, there are true vascular tumours which are of intermediate grade and frank malignant tumours.

HAEMANGIOMA. Haemangiomas are quite common lesions, especially in infancy and childhood. The most common site is the skin of the face. Amongst the various clinical and histologic types, three important forms are described below.

Capillary haemangioma. These are the most common type. Clinically, they appear as small or large, flat or slightly elevated, red to purple, soft and lobulated lesions, varying in size from a few millimeters to a few centimeters in diameter. They may be present at birth or appear in early childhood. Strawberry birthmarks and 'portwine mark' are some good examples. The common sites are the skin, subcutaneous tissue and mucous membranes of oral cavity and lips. Less common sites are internal visceral organs like liver, spleen and kidneys.

Microscopically, capillary haemangiomas are well defined but unencapsulated lobules. These lobules are composed of capillary-sized, thin-walled, blood-filled vessels. These vessels are lined by single layer of plump endothelial cells surrounded by a layer of pericytes. The vessels are separated by some connective tissue stroma (Fig. 23.18) (COLOUR PLATE IX: CL 34).

**FIGURE 23.18**

Capillary haemangioma of the skin. There are capillaries lined by plump endothelial cells and containing blood. The intervening stroma consists of scant connective tissue.

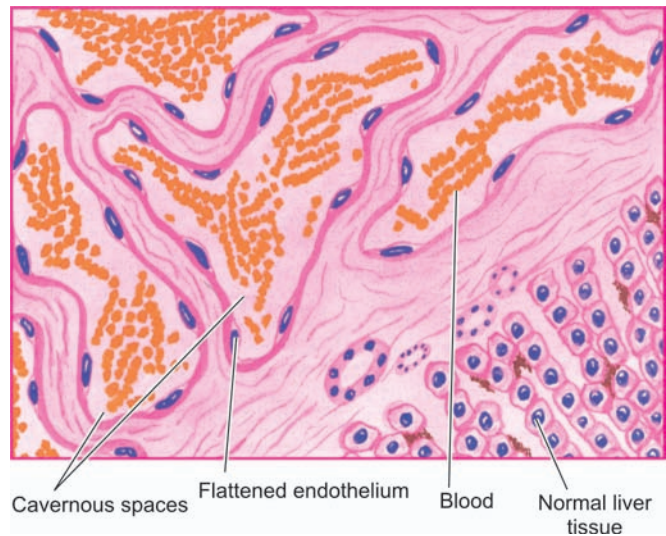
Many of the capillary haemangiomas regress spontaneously within a few years.

Cavernous haemangioma. Cavernous haemangiomas are single or multiple, discrete or diffuse, red to blue, soft and spongy masses. They are often 1 to 2 cm in diameter. They are most common in the skin (especially of the face and neck); other sites are mucosa of the oral cavity, stomach and small intestine, and internal viscera like the liver and spleen.

Microscopically, cavernous haemangiomas are composed of thin-walled cavernous vascular spaces, filled partly or completely with blood. The vascular spaces are lined by flattened endothelial cells. They are separated by scanty connective tissue stroma (Fig. 23.19).

Cavernous haemangiomas rarely involute spontaneously.

Granuloma pyogenicum. Granuloma pyogenicum is also referred to as *haemangioma of granulation tissue type*. True to its name, it appears as exophytic, red granulation tissue just like a nodule, commonly on the skin and mucosa of gingiva or oral cavity. Pregnancy tumour or *granuloma gravidarum* is a variant occurring on the gingiva during pregnancy and regresses after delivery. Granuloma pyogenicum often develops following trauma and is usually 1 to 2 cm in diameter.

**FIGURE 23.19**

Cavernous haemangioma of the liver. The vascular spaces are large, dilated, many containing blood, and are lined by flattened endothelial cells. Scanty connective tissue stroma is seen between the cavernous spaces.

Microscopically, it shows proliferating capillaries similar to capillary haemangioma but the capillaries are separated by abundant oedema and inflammatory infiltrate, thus resembling inflammatory granulation tissue.

LYMPHANGIOMA. Lymphangiomas are lymphatic counterparts of vascular haemangiomas. Lymphangiomas are congenital lesions which are classified as capillary, cavernous and cystic hygroma. Combinations are also often seen.

Capillary lymphangioma. It is also called as lymphangioma simplex. It is a small, circumscribed, slightly elevated lesion measuring 1 to 2 cm in diameter. The common locations are the skin of head and neck, axilla and mucous membranes. Rarely, these may be found in the internal organs.

Microscopically, capillary lymphangioma is composed of a network of endothelium-lined, capillary-sized spaces containing lymph and often separated by lymphoid aggregates.

Cavernous lymphangioma. It is more common than the capillary type. The common sites are in the region of head and neck or axilla. A large cystic variety called *cystic hygroma* occurs in the neck producing gross deformity in the neck.

Microscopically, cavernous lymphangioma consists of large dilated lymphatic spaces lined by flattened endothelial cells and containing lymph. Scant intervening stromal connective tissue is present (Fig. 23.20). These lesions, though benign, are often difficult to remove due to infiltration into adjacent tissues.

GLOMUS TUMOUR (GLOMANGIOMA). Glomus tumour is an uncommon true benign tumour arising from contractile glomus cells that are present in the arteriovenous shunts (Sucquet-Hoxer anastomosis). These tumours are found most often in the dermis of the fingers or toes under a nail; other sites are mucosa of the stomach and nasal cavity. These lesions are characterised by extreme pain. They may be single or multiple, small, often less than 1 cm in diameter, flat or slightly elevated, red-blue, painful nodules.

Microscopically, the tumours are composed of small blood vessels lined by endothelium and surrounded by aggregates, nests and masses of glomus cells. The glomus cells are round to cuboidal cells with scanty cytoplasm. The intervening connective tissue stroma contains some non-myelinated nerve fibres (Fig. 23.21).

HAEMANGIOENDOTHELIOMA. Haemangioendothelioma is a true tumour of endothelial cells, the

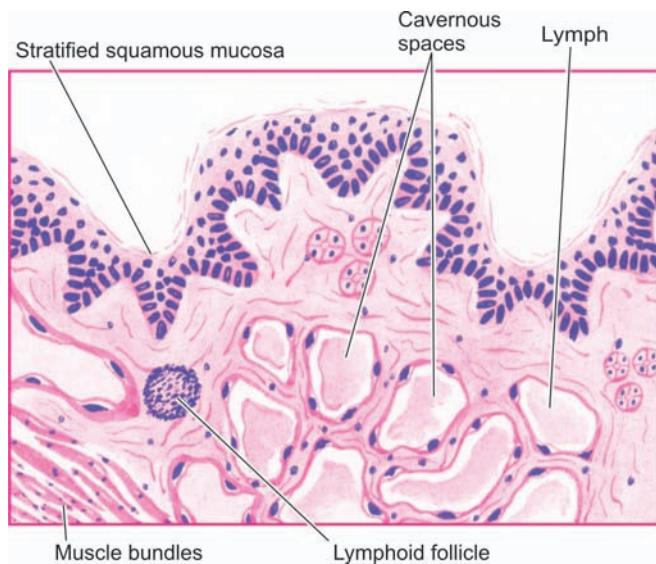


FIGURE 23.20

Cavernous lymphangioma of the tongue. Large cystic spaces lined by the flattened endothelial cells and containing lymph are present. Stroma shows scattered collection of lymphocytes.

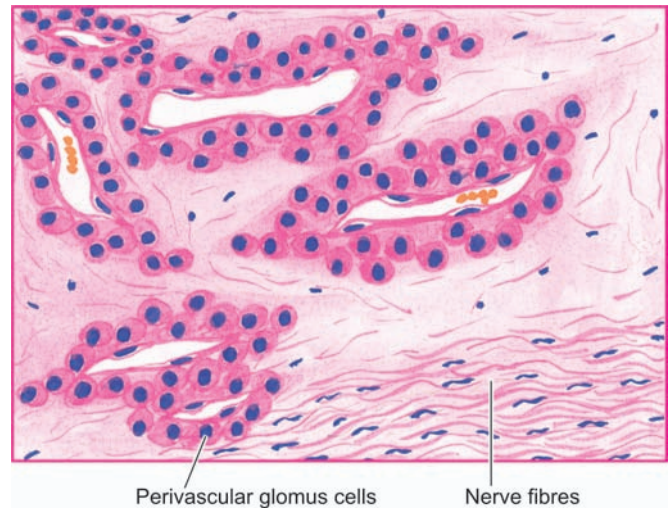


FIGURE 23.21

Glomus tumour. There are blood-filled vascular channels lined by endothelial cells and surrounded by nests and masses of glomus cells.

behaviour of which is intermediate between the haemangioma and haemangiosarcoma. It is found most often in the skin and subcutaneous tissues, and liver of children. *Haemangioblastoma* is the term used for similar tumour occurring in the cerebellum.

Grossly, the tumour is usually well-defined, grey red, polypoid mass.

Microscopically, there is active proliferation of endothelial cells forming several layers around the blood vessels so that vascular lumina are difficult to identify.

ANGIOSARCOMA. Also known as haemangiosarcoma and malignant haemangioendothelioma, it is a malignant vascular tumour occurring most frequently on the skin, subcutaneous tissue, liver, spleen, bone, lung and retroperitoneal tissues. It can occur in both sexes and at any age. *Hepatic angiosarcomas* are of special interest in view of their association with carcinogens like polyvinyl chloride, arsenical pesticides and radioactive contrast medium, thorotrast.

Grossly, the tumours are usually bulky, pale grey white, firm masses with poorly-defined margins. Areas of haemorrhage, necrosis and central softening are frequently present.

Microscopically, the tumours may be well-differentiated masses of proliferating endothelial cells around

well-formed vascular channels, to poorly differentiated lesions composed of plump, anaplastic and pleomorphic cells in solid clusters with poorly identifiable vascular channels.

These tumours invade locally and may have distant metastases in lungs and other organs. *Lymphangiosarcoma* is a histologically similar tumour occurring in obstructive lymphoedema of long duration.

TERATOMA

Teratomas are complex tumours composed of tissues derived from more than one of the three germ cell layers—endoderm, mesoderm and ectoderm. They are most commonly seen in gonads of males and females (i.e. testis and ovaries). Testicular teratomas are more common in infants and children while ovarian teratomas are more frequent during their active reproductive life. Sometimes, teratomas may be found in combination with other germ cell tumours. AFP levels are slightly raised in teratomas.

Teratomas are classified into 3 types:

1. Mature (differentiated) teratoma
2. Immature teratoma
3. Teratoma with malignant transformation.

Grossly, most *testicular teratomas* are large, grey-white masses enlarging the involved testis. Cut surface shows characteristic variegated appearance—grey-white solid areas, cystic and honey-combed areas,

and foci of cartilage and bone (Fig. 23.22, A). Dermoid tumours commonly seen in the ovaries are rare in testicular teratomas.

The vast majority of *ovarian teratomas* on the other hand, are benign and cystic and have the predominant ectodermal elements, often termed clinically as dermoid cyst. Benign cystic teratoma or dermoid cyst is characteristically a unilocular cyst, 10–15 cm in diameter, usually lined by skin and hence its name. On sectioning, the cyst is filled with paste-like sebaceous secretions and desquamated keratin admixed with masses of hair. The cyst wall is thin and opaque grey-white. Generally, in one area of the cyst wall, a solid prominence is seen (Rokitansky's protuberance) where tissue elements such as tooth, bone, cartilage and various other odd tissues are present (Fig. 23.23, A).

Microscopically, the three categories of teratomas show different appearances:

1. Mature (differentiated) teratoma. *Mature testicular teratoma* is composed of disorderly mixture of a variety of well-differentiated structures such as cartilage, smooth muscle, intestinal and respiratory epithelium, mucous glands, cysts lined by squamous and transitional epithelium, neural tissue, fat and bone. This type of mature or differentiated teratoma of the testis is the most common, seen more frequently in infants and children and has favourable prognosis. But similar mature and benign-appearing tumour in

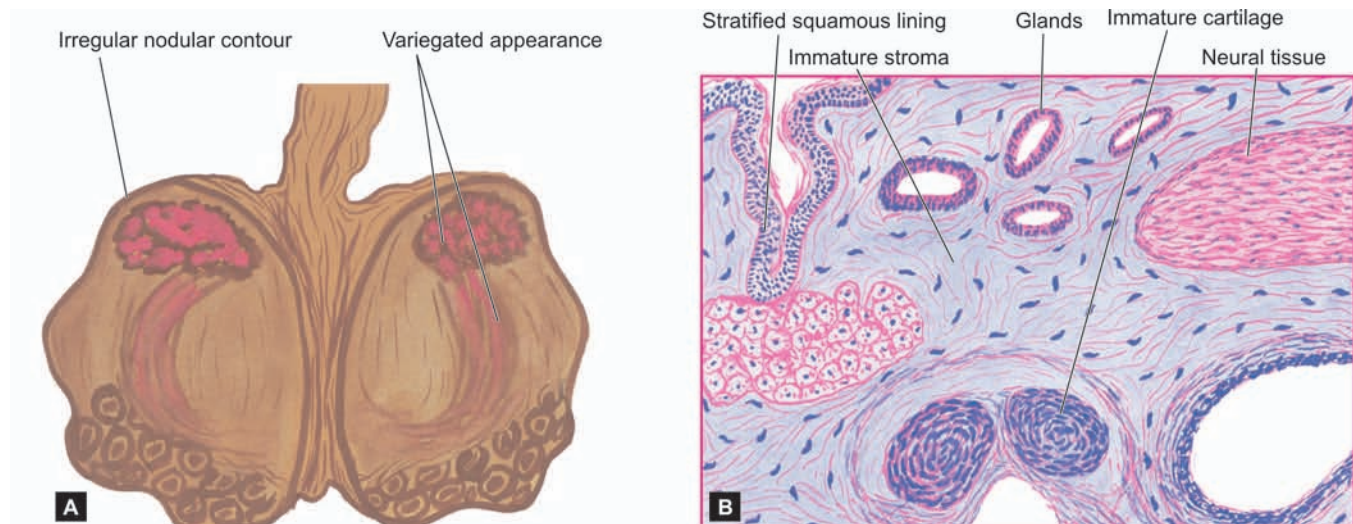
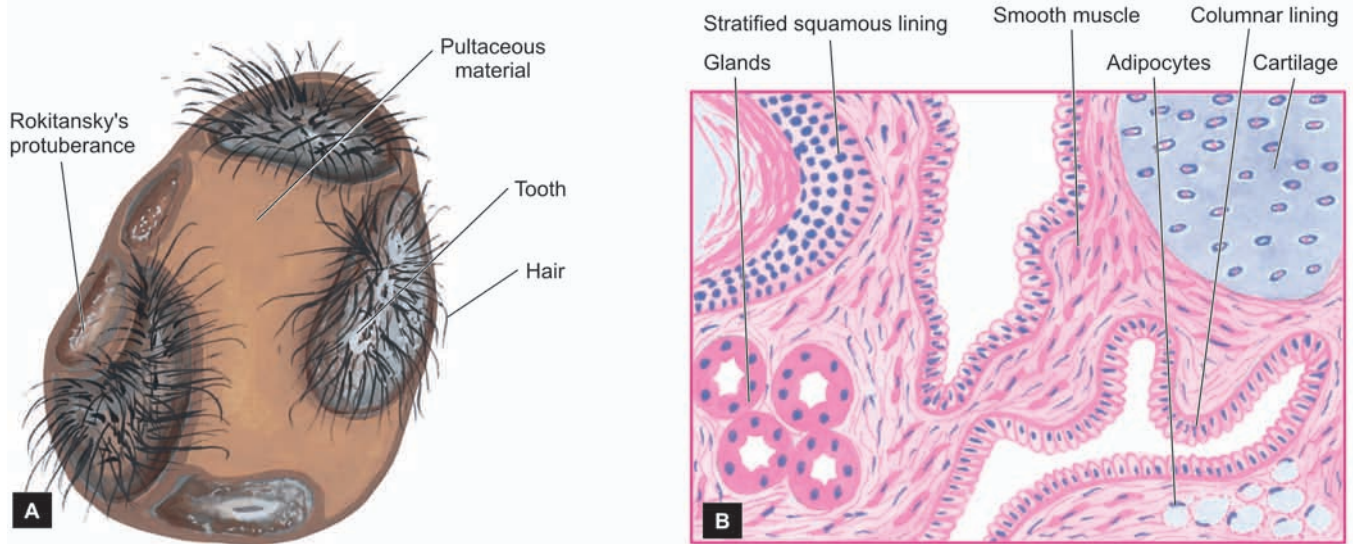


FIGURE 23.22

Immature teratoma. A, Typical gross appearance on cut section. The tumour produces an irregular, nodular enlargement of the testis, while the sectioned surface shows characteristic variegated appearance. B, Microscopy shows incompletely differentiated tissue elements.

**FIGURE 23.23**

Benign cystic teratoma (dermoid cyst) of the ovary A, Gross appearance. B, Microscopy shows characteristic lining of the cyst wall by epidermis and its appendages.

adults is invariably associated with small hidden foci of immature elements so that their clinical course in adults is unpredictable (Fig. 23.22,B). It is believed that all testicular teratomas in the adults are malignant.

In the case of *mature ovarian teratoma*, the most prominent feature is the lining of the cyst wall by stratified squamous epithelium and its adnexal structures such as sebaceous glands, sweat glands and hair follicles (Fig. 23.23,B). Though ectodermal derivatives are most prominent features, tissues of mesodermal and endodermal origin are commonly present as in mature testicular teratomas. Thus, viewing a benign teratoma in different microscopic fields reveals a variety of mature differentiated tissue elements, producing *kaleidoscopic patterns*.

2. Immature teratoma. Immature teratoma is composed of incompletely differentiated and primitive or embryonic tissues along with some mature elements. Primitive or embryonic tissue commonly present are poorly-formed cartilage, mesenchyme, neural tissues, abortive eye, intestinal, respiratory tissue elements etc (Fig. 23.22,B). Mitoses are usually frequent.

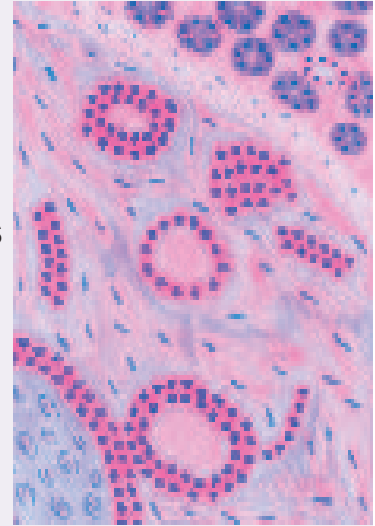
3. Teratoma with malignant transformation. This is an extremely rare form of teratoma in which one or more of the tissue elements show malignant transformation. Such malignant change resembles morphologically with typical malignancies in other organs and tissues and commonly includes rhabdomyosarcoma, squamous cell carcinoma and adenocarcinoma.



Selected Topics from Systemic Pathology

SECTION CONTENTS

- CHAPTER 24: Atherosclerosis, IHD
- CHAPTER 25: Rheumatic Heart Disease, Bacterial Endocarditis
- CHAPTER 26: Pneumonias, Lung Cancer
- CHAPTER 27: Diseases of Oral Cavity & Salivary Glands
- CHAPTER 28: Jaundice, Viral Hepatitis, Cirrhosis
- CHAPTER 29: Glomerulonephritis, Pyelonephritis
- CHAPTER 30: Systemic Hypertension
- CHAPTER 31: Diabetes Mellitus
- CHAPTER 32: Common Diseases of Bones



24

Atherosclerosis, IHD

ATHEROSCLEROSIS

Atherosclerosis is a specific form of arteriosclerosis affecting primarily the intima of large and medium-sized muscular arteries and is characterised by fibrofatty plaques or atheromas. The term atherosclerosis is derived from *athero*-(meaning porridge) referring to the soft lipid-rich material in the centre of atheroma, and *sclerosis* (scarring) referring to connective tissue in the plaques. Atherosclerosis is the commonest and the most important of the arterial diseases. Though any large and medium-sized artery may be involved in atherosclerosis, the most commonly affected are the aorta, the coronary and the cerebral arterial systems. Therefore, the major clinical syndromes resulting from ischaemia due to atherosclerosis are the myocardial infarcts (*heart attacks*) and the cerebral infarcts (*strokes*); other less common sequelae are peripheral vascular disease, aneurysmal dilatation due to weakened arterial wall, chronic ischaemic heart disease, ischaemic encephalopathy and mesenteric occlusion.

Etiology

Atherosclerosis is widely prevalent in industrialised countries. However, majority of the incidences quoted in the literature are based on the major clinical syndromes produced by it, the most important interpretation being that death from myocardial infarction is related to underlying atherosclerosis. Cardiovascular disease, mostly related to atherosclerotic coronary heart disease or ischaemic heart disease (IHD) is the most common cause of death in the developed countries of the world.

Extensive epidemiologic investigations on living populations have revealed a number of **risk factors** which are associated with increased risk of developing clinical atherosclerosis. Often, these risk factors are acting in combination rather than singly.

These risk factors are divided into two groups (Table 24.1):

I. Major risk factors. These are further considered under 2 headings:

TABLE 24.1: Risk Factors in Atherosclerosis.

I. MAJOR RISK FACTORS	II. MINOR RISK FACTORS
A) Constitutional	1. Environmental influences
1. Age	2. Obesity
2. Sex	3. Hormones: oestrogen deficiency, oral contraceptives
3. Genetic factors	4. Physical inactivity
4. Familial and racial factors	5. Stressful life
B) Acquired	6. Infections (<i>C. pneumoniae</i> , Herpesvirus, CMV)
1. Hyperlipidaemia	7. Homocystinuria
2. Hypertension	8. Role of alcohol
3. Diabetes mellitus	
4. Smoking	

A) *Major constitutional risk factors*: These are non-modifiable major risk factors that includes: increasing age, male sex, genetic abnormalities, and familial and racial predisposition.

B) *Major acquired risk factors*: This includes major risk factors which can be controlled and includes: hyperlipidaemia, hypertension, diabetes mellitus and smoking.

II. Minor risk factors. This includes a host of factors whose role in atherosclerosis is minimal, and in some cases, even uncertain.

Apparently, a combination of etiologic risk factors have additive effect in producing the lesions of atherosclerosis.

MAJOR CONSTITUTIONAL RISK FACTORS

Age, sex and genetic influences do affect the appearance of lesions of atherosclerosis.

1. AGE. Atherosclerosis is an age-related disease. Though early lesions of atherosclerosis may be present in childhood, clinically significant lesions are found with increasing age. Fully-developed atheromatous plaques usually appear in the 4th decade and beyond. Evidence in support comes from the high death rate from IHD in this age group.

2. SEX. The incidence and severity of atherosclerosis are more in men than in women. The prevalence of atherosclerotic IHD is about three times higher in men in 4th decade than in women and the difference slowly declines with age but remains higher at all ages in men. The lower incidence of IHD in women, especially in premenopausal age, is probably due to high levels of oestrogen and high-density lipoproteins, both of which have anti-atherogenic influence.

3. GENETIC FACTORS. Genetic factors play a significant role in atherogenesis. Hereditary genetic

derangements of lipoprotein metabolism predispose the individual to high blood lipid level and familial hypercholesterolaemia.

4. FAMILIAL AND RACIAL FACTORS: The familial predisposition to atherosclerosis may be related to other risk factors like diabetes, hypertension and hyperlipoproteinaemia. Racial differences too exist; Blacks have generally less severe atherosclerosis than Whites.

MAJOR ACQUIRED RISK FACTORS

There are four major acquired risk factors in atherogenesis—hyperlipidaemia, hypertension, cigarette smoking and diabetes mellitus.

1. HYPERLIPIDAEMIA. Virchow in 19th century first identified cholesterol crystals in the atherosclerotic lesions. Since then, extensive information on lipoproteins and their role in atherosclerotic lesions has been gathered. It is now well established that hypercholesterolaemia has directly proportionate relationship with atherosclerosis and IHD.

The main lipids in blood are cholesterol (desirable normal 140-200 mg/dl, borderline high 240 mg/dl) and triglycerides (below 160 mg/dl). An elevation of serum cholesterol levels above 260 mg/dl in men and women between 30 and 50 years of age has three times higher risk of developing IHD as compared with people with serum cholesterol levels within normal limits. The concentration of cholesterol in the serum reflects the concentrations of different lipoproteins in the serum. The lipoproteins are divided into classes according to the density of solvent in which they remain suspended on centrifugation at high speed. The major classes of lipoprotein particles are *chylomicrons*, *very-low density lipoproteins* (VLDL), *low-density lipoproteins* (LDL), and *high-density lipoproteins* (HDL). Lipids are insoluble in blood and therefore are carried in circulation and across the cell membrane by carrier proteins called *apoproteins*. Apoprotein surrounds the lipid for carrying it, different apoproteins being named by letter A, B, C, D etc while their subfractions are numbered serially. The major fractions of lipoproteins and their varying effects on atherosclerosis and IHD are as under (Table 24.2):

■ *Low-density lipoprotein* (LDL) is richest in cholesterol and has the maximum association with atherosclerosis.

■ *Very-low-density lipoprotein* (VLDL) carries much of the triglycerides and has less marked effect than LDL.

■ *High-density lipoprotein* (HDL) is protective 'good cholesterol' against atherosclerosis.

Many studies have demonstrated the harmful effect of diet containing larger quantities of saturated fats (e.g.

TABLE 24.2: Fractions of Lipoproteins in Serum.

CLASSES	SITES OF SYNTHESIS	NORMAL SERUM LEVELS	ROLE IN ATHEROSCLEROSIS
1. <i>HDL cholesterol</i>	Liver, intestine	> 40 mg/dl	Protective
2. <i>LDL cholesterol</i>	Liver	< 130 mg/dl	Maximum
3. <i>VLDL triglycerides</i>	Intestine, liver	< 160 mg/dl	Less marked
4. <i>Chylomicrons</i>	Liver, intestine, macrophage	—	Indirect

in eggs, meat, milk, butter etc) which raise the plasma cholesterol level. This type of diet is consumed more often by the affluent societies who are at greater risk of developing atherosclerosis. On the contrary, a diet low in saturated fats and high in poly-unsaturated fats and having omega-3 fatty acids (e.g. in fish, fish oils etc) lowers the plasma cholesterol levels. Aside from lipid-rich diet, high intake of the total number of calories from carbohydrates, proteins, alcohol and sweets has adverse effects.

How hypercholesterolaemia and various classes of lipoproteins produce atherosclerosis is described under 'pathogenesis'.

2. HYPERTENSION. Hypertension is the other major risk factor in the development of atherosclerotic IHD and cerebrovascular disease. It acts probably by mechanical injury to the arterial wall due to increased blood pressure. A systolic pressure of over 160 mmHg or a diastolic pressure of over 95 mmHg is associated with five times higher risk of developing IHD than in people with blood pressure within normal range (140/90 mmHg or less).

3. SMOKING. The extent and severity of atherosclerosis are much greater in smokers than in non-smokers. Cigarette smoking is associated with higher risk of atherosclerotic IHD and sudden cardiac death. Men who smoke a pack of cigarettes a day are 3-5 times more likely to die of IHD than non-smokers. The increased risk and severity of atherosclerosis in smokers is due to reduced level of HDL and accumulation of carbon monoxide in the blood that produces carboxyhaemoglobin and eventually hypoxia in the arterial wall favouring atherosclerosis.

4. DIABETES MELLITUS. Clinical manifestations of atherosclerosis are far more common and develop at an early age in people with both insulin-dependent and non-insulin dependent diabetes mellitus. The risk of developing IHD is doubled, tendency to develop cerebrovascular disease is high, and frequency to develop gangrene of foot is about 100 times increased.

The causes of increased severity of atherosclerosis are complex and numerous which include increased aggregation of platelets, increased LDL and decreased HDL.

MINOR RISK FACTORS

There are a number of less important and minor risk factors having some role in the etiology of atherosclerosis. These are as under:

1. Higher incidence of atherosclerosis in developed countries and low prevalence in underdeveloped countries, suggesting the role of *environmental influences*.
2. *Obesity*, if the person is overweight by 20% or more, is associated with increased risk.
3. Use of *exogenous hormones* (e.g. oral contraceptives) by women or *endogenous oestrogen deficiency* (e.g. in post-menopausal women) has been shown to have increased risk of developing myocardial infarction or stroke.
4. *Physical inactivity* and lack of exercise are associated with the risk of developing atherosclerosis and its complications.
5. *Stressful life style*, termed as type A behaviour pattern, characterised by aggressiveness, competitive drive, ambitiousness and a sense of urgency, is associated with enhanced risk of IHD compared with type B behaviour of relaxed and happy-go-lucky type.
6. Recently role of *infections*, particularly of *Chlamydia pneumoniae* and viruses such as herpesvirus and cytomegalovirus, has been found in coronary atherosclerotic lesions. Possibly, infections may be acting in combination with some other factors.
7. Patients with *homocystinuria*, an uncommon inborn error of metabolism, have been reported to have early atherosclerosis and coronary artery disease.
8. Moderate consumption of *alcohol* appears to have slightly beneficial effect by raising the level of HDL cholesterol and by causing vasodilatation but the matter remains controversial.

Pathogenesis

As stated above, atherosclerosis is not caused by a single etiologic factor but is a multifactorial disease whose

exact pathogenesis is still not known. A number of theories have been proposed since the times of Virchow.

■ **Insudation hypothesis.** The concept hypothesised by Virchow in 1856 that atherosclerosis is a form of cellular proliferation of the intimal cells resulting from increased imbibing of lipids from the blood came to be called the 'lipid theory', currently known as 'response to injury hypothesis' and is now-a-days the most widely accepted theory.

■ **Encrustation hypothesis.** The proposal put forth by Rokitansky in 1852 that atheroma represented a form of encrustation on the arterial wall from the components in the blood forming thrombi composed of platelets, fibrin and leucocytes, was named as 'encrustation theory' or 'thrombogenic theory'. Since currently it is believed that encrustation or thrombosis is not the sole factor in atherogenesis but the components of thrombus (platelets, fibrin and leucocytes) have a role in atheromatous lesions, this theory has now been incorporated into the foregoing recent theory of response to injury.

Thus, there is no consensus regarding the origin and progression of lesion of atherosclerosis. The role of four key factors—arterial smooth muscle cells, endothelial cells, blood monocytes and hyperlipidaemia, is accepted by all. However, the areas of disagreement exist in the mechanism and sequence of events involving these factors in initiation, progression and complications of disease. Currently, pathogenesis of atherosclerosis is explained on the basis of the following two theories:

1. Reaction-to-injury hypothesis, first described in 1973, and modified in 1986 and 1993 by Ross.
2. Monoclonal theory, based on neoplastic proliferation of smooth muscle cells, postulated by Benditt and Benditt in 1973.

1. REACTION-TO-INJURY HYPOTHESIS. This theory is most widely accepted and incorporates aspects of two older historical theories of atherosclerosis—the lipid theory of Virchow and thrombogenic (encrustation) theory of Rokitansky.

■ The *original response to injury theory* was first described in 1973 according to which the initial event in atherogenesis was considered to be endothelial injury followed by smooth muscle cell proliferation so that the early lesions, according to this theory, consist of smooth muscle cells mainly.

■ The *modified response-to-injury hypothesis* described subsequently in 1993 implicates lipoprotein entry into the intima as the initial event followed by lipid accumulation in the macrophages (foam cells now) which according to modified theory, are believed to be the dominant cells in early lesions.

Both these theories—original and modified, have attracted support and criticism. However, following is the generally accepted role of key components involved in atherogenesis, diagrammatically illustrated in Fig. 24.1.

i) **Endothelial injury.** It has been known for many years that endothelial injury is the initial triggering event

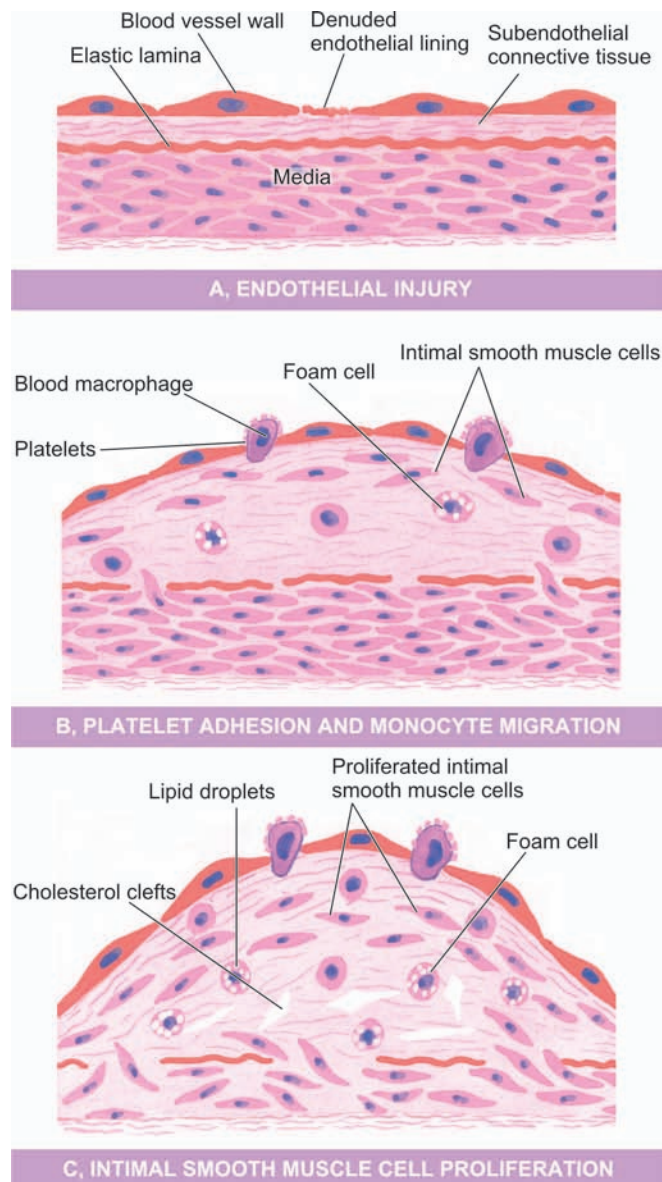


FIGURE 24.1

Diagrammatic representation of pathogenesis of atherosclerosis as explained by 'reaction-to-injury' hypothesis. A, Endothelial injury. B, Adhesion of platelets and migration of blood monocytes from blood stream. C, Smooth muscle cell proliferation into the intima and ingrowth of new blood vessels.

in the development of lesions of atherosclerosis. Actual endothelial denudation is not an essential requirement, but endothelial dysfunction may initiate the sequence of events. Numerous causes ascribed to endothelial injury in experimental animals are: mechanical trauma, haemodynamic forces, immunological and chemical mechanisms, metabolic agent as chronic hyperlipidaemia, homocystine, circulating toxins from systemic infections, viruses, hypoxia, radiation, carbon monoxide and tobacco products.

In man, two of the major risk factors which act together to produce endothelial injury are: *haemodynamic stress from hypertension and chronic hyperlipidaemia*. The role of haemodynamic forces in causing endothelial injury is further supported by the distribution of atheromatous plaques at points of bifurcation or branching of blood vessels which are under greatest shear stress.

ii) Intimal smooth muscle cell proliferation. Endothelial injury causes adherence, aggregation and platelet release reaction at the site of exposed subendothelial connective tissue. Proliferation of intimal smooth muscle cells is stimulated by various mitogens released from platelets adherent at the site of endothelial injury and monocytes, the most important of which is platelet-derived growth factor (PDGF); others are fibroblast growth factor (FGF), and transforming growth factor- α (TGF- α). Smooth muscle cell proliferation is also facilitated by biomolecules such as nitric oxide and *endothelin* released from endothelial cells. Intimal proliferation of smooth muscle cells is accompanied by synthesis of matrix proteins—collagen, elastic fibre proteins and proteoglycans.

iii) Role of blood monocytes. Though blood monocytes do not possess receptors for normal LDL, LDL does appear in the monocyte cytoplasm to form foam cell by mechanism illustrated in Fig. 24.2. Plasma LDL on entry into the intima undergoes oxidation. The 'oxidised LDL' so formed in the intima performs the following all-important functions on monocytes and endothelium:

- For monocytes, oxidised LDL acts to attract, proliferate, immobilise and activate them as well as is readily taken up by scavenger receptor on the monocyte to transform it to a lipid-laden foam cell.
- For endothelium, oxidised LDL is cytotoxic.

Death of foam cell by apoptosis releases lipid to form lipid core of plaque.

iv) Role of hyperlipidaemia. As stated already, chronic hyperlipidaemia in itself may initiate endothelial injury

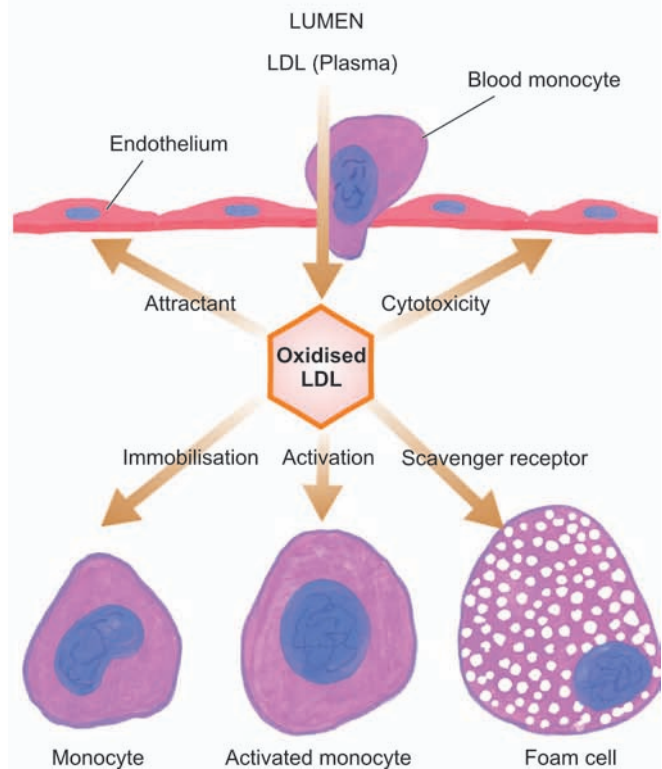


FIGURE 24.2

Mechanism of foam cell formation.

and dysfunction by causing increased permeability. Secondly, increased serum concentration of LDL and VLDL promotes formation of foam cells, while high serum concentration of HDL has anti-atherogenic effect.

v) Thrombosis. As apparent from the foregoing, endothelial injury exposes subendothelial connective tissue resulting in formation of small platelet aggregates at the site and causing proliferation of smooth muscle cells. This causes mild inflammatory reaction which together with foam cells is incorporated into the atheromatous plaque. The lesions enlarge by attaching fibrin and cells from the blood so that thrombus becomes a part of atheromatous plaque.

2. MONOCLONAL HYPOTHESIS. This hypothesis is based on the postulate that proliferation of smooth muscle cells is the primary event and that this proliferation is monoclonal in origin similar to cellular proliferation in neoplasms (e.g. in uterine leiomyoma, Chapter 21). The evidence cited in support of monoclonal hypothesis is the observation on proliferated smooth muscle cells in atheromatous plaques which have only one of the two forms of glucose-6-phosphate dehydrogenase

(G6PD) isoenzymes, suggesting monoclonality in origin. The monoclonal proliferation of smooth muscle cells in atherosclerosis may be initiated by mutation caused by exogenous chemicals (e.g. cigarette smoke), endogenous metabolites (e.g. lipoproteins) and some viruses (e.g. Marek's disease virus in chickens, herpesvirus).

PATHOLOGIC CHANGES

Early lesions in the form of diffuse intimal thickening, fatty streaks and gelatinous lesions are often the forerunners in the evolution of atherosclerotic lesions (Fig. 24.3,A). However, the clinical disease states due to luminal narrowing in atherosclerosis are caused by fully developed atheromatous plaques and complicated plaques (Fig. 24.3,B, C).

1. FATTY STREAKS AND DOTS. Fatty streaks and dots on the intima by themselves are harmless but may be the precursor lesions of atheromatous plaques. They are seen in all races of the world and begin to appear in the first year of life. However, they are uncommon in older persons and are probably absorbed. They are especially prominent in the aorta and other major arteries, more often on the posterior wall than the anterior wall.

Grossly, the lesions may appear as flat or slightly elevated and yellow. They may be either in the form of small, multiple dots, about 1 mm in size, or in the form of elongated, beaded streaks.

Microscopically, fatty streaks lying under the endothelium are composed of closely-packed foam cells, lipid-containing elongated smooth muscle cells and a few lymphoid cells. Small amount of extracellular lipid, collagen and proteoglycans are also present.

2. GELATINOUS LESIONS. Gelatinous lesions develop in the intima of the aorta and other major arteries in the first few months of life. Like fatty streaks, they may also be precursors of plaques. They are round or oval, circumscribed grey elevations, about 1 cm in diameter.

Microscopically, gelatinous lesions are foci of increased ground substance in the intima with thinned overlying endothelium.

3. ATHEROMATOUS PLAQUES. A fully developed atherosclerotic lesion is called atheromatous plaque, also called *fibrous plaque*, *fibrofatty plaque* or *atheroma*. Unlike fatty streaks, atheromatous plaques are selective in different geographic locations and races and are seen in advanced age. These lesions may develop from progression of early lesions of the atherosclerosis described above. *Most often and most severely affected is the abdominal aorta*, though smaller

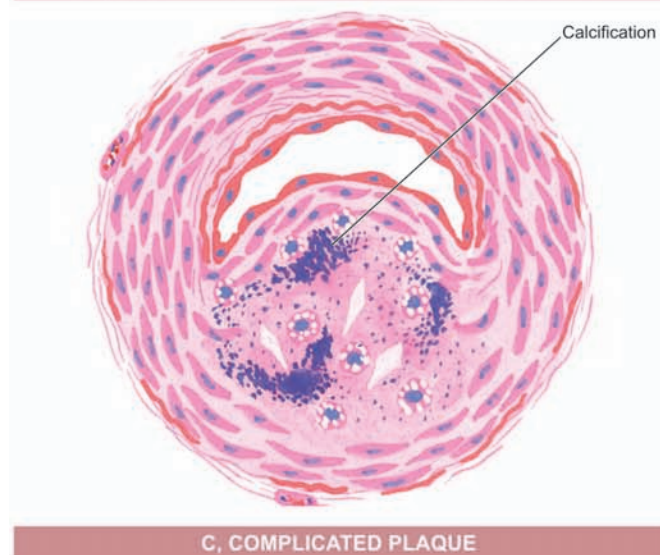
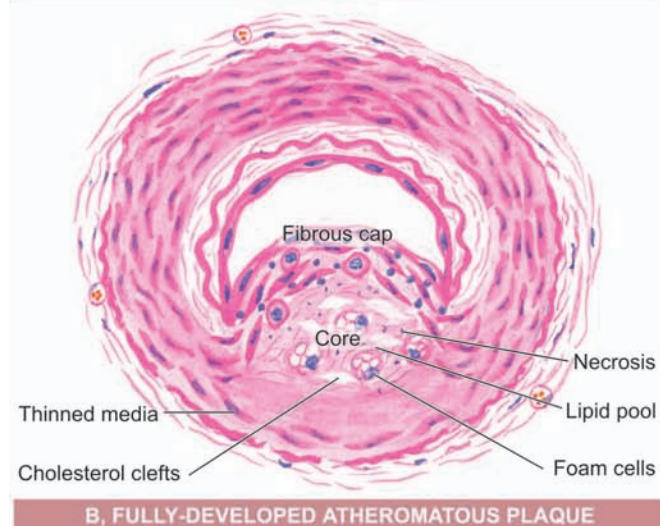
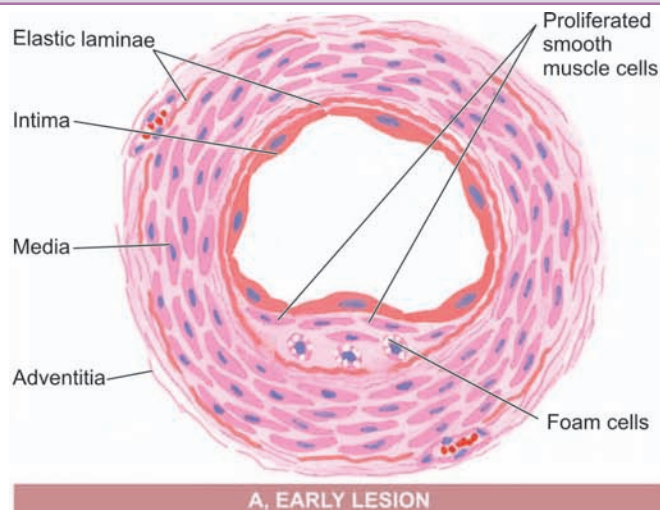


FIGURE 24.3

Evolution of lesions in atherosclerosis. For details, see the text.

lesions may be seen in descending thoracic aorta and aortic arch. The major branches of the aorta around the ostia are often severely involved, especially the iliac, femoral, carotid, coronary, and cerebral arteries. **Grossly**, atheromatous plaques are white to yellowish-white lesions, varying in diameter from 1-2 cm and raised on the surface by a few millimetres to a centimetre in thickness (Fig. 24.4,A). Cut section of the plaque reveals the luminal surface as a firm, white *fibrous cap* and a *central core* composed of yellow to yellow-white, soft, porridge-like material and hence the name atheroma.

Microscopically, the appearance of plaque varies depending upon the age of the lesion. However, the following features are invariably present (Fig. 24.4,B):

- The superficial luminal part of *fibrous cap* is covered by endothelium, and is composed of smooth muscle cells, dense connective tissue and extracellular matrix containing proteoglycans and collagen.
- The *cellular area* under the fibrous cap is comprised by a mixture of macrophages, foam cells, lymphocytes and a few smooth muscle cells which may contain lipid.
- The deeper *central soft core* consists of extracellular lipid material, cholesterol clefts, fibrin, necrotic debris and lipid-laden foam cells.

■ In older and *more advanced lesions*, the collagen in the fibrous cap may be dense and hyalinised, smooth muscle cells may be atrophic and foam cells are fewer.

4. COMPLICATED PLAQUES. Various pathologic changes that occur in fully-developed atheromatous plaques are called the complicated lesions. These account for the most serious harmful effects of atherosclerosis and even death. These changes include calcification, ulceration, thrombosis, haemorrhage and aneurysmal dilatation. It is not uncommon to see more than one form of complication in a plaque.

i) Calcification. Calcification occurs more commonly in advanced atheromatous plaques, especially in the aorta and coronaries. The diseased intima cracks like an egg-shell when the vessel is incised and opened.

Microscopically, the calcium salts are deposited in the vicinity of necrotic area and in the soft lipid pool deep in the thickened intima (**COLOUR PLATE II: CL 6**). This form of atherosclerotic *intimal* calcification differs from Mönckeberg's medial calcific arteriosclerosis that affects only the tunica media.

ii) Ulceration. The layers covering the soft pul-taceous material of an atheroma may ulcerate as a result of haemodynamic forces or mechanical trauma. This results in discharge of emboli composed of lipid

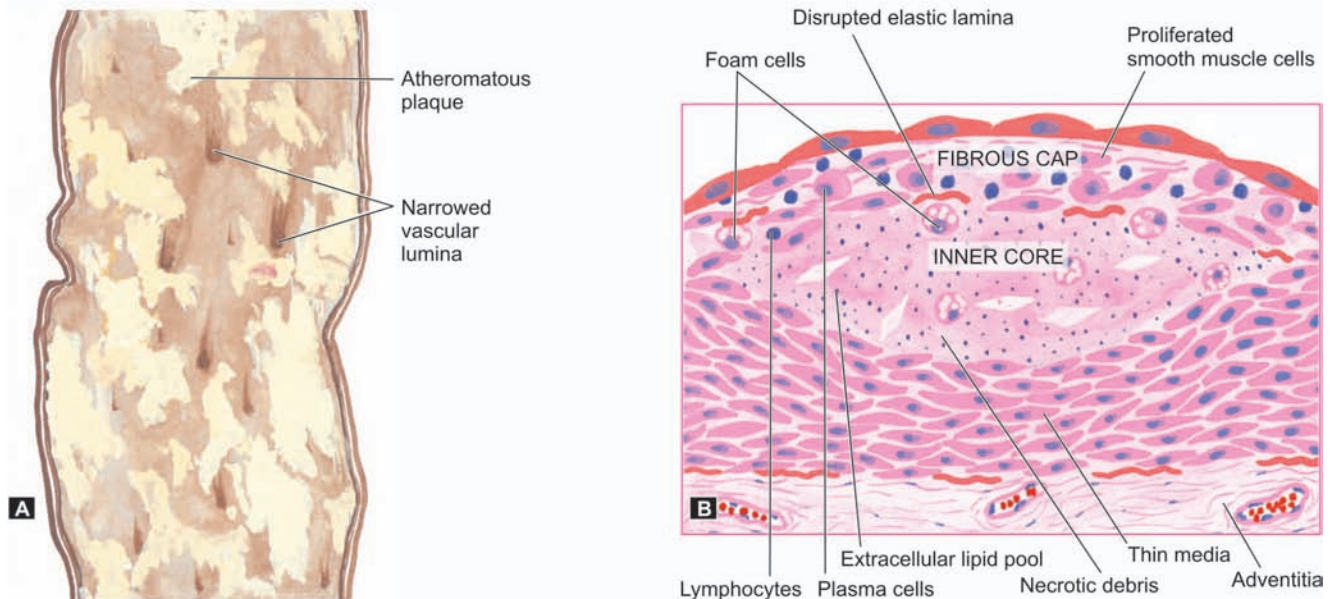


FIGURE 24.4

Structure of a fully-developed atheroma. A, Inner surface of opened abdominal aorta shows a variety of atheromatous lesions. While some are raised fibrous plaques, others are ulcerated with superimposed thrombosis. Orifices of some of the branches are narrowed by the atherosclerotic process. B, Diagrammatic view of the histologic appearance of a fully-developed atheroma.

material and debris into the blood stream, leaving a shallow, ragged ulcer with yellow lipid debris in the base of the ulcer. Occasionally, atheromatous plaque in a coronary artery may suddenly rupture into the arterial lumen forcibly and cause thromboembolic occlusion.

iii) Thrombosis. The ulcerated plaque and the areas of endothelial damage are vulnerable sites for formation of superimposed thrombi. These thrombi may get dislodged to become emboli and lodge elsewhere in the circulation, or may get organised and incorporated into the arterial wall as mural thrombi. Mural thrombi may become occlusive thrombi which may subsequently recanalise.

iv) Haemorrhage. Intimal haemorrhage may occur in an atheromatous plaque either from the blood in the vascular lumen through an ulcerated plaque, or from rupture of thin-walled capillaries that vascularise the atheroma from adventitial vasa vasorum. Haemorrhage is particularly a common complication in coronary arteries. The haematoma formed at the site contains numerous haemosiderin-laden macrophages.

v) Aneurysm formation. Though atherosclerosis is primarily an intimal disease, advanced lesions are associated with secondary changes in the media and adventitia. The changes in media include atrophy and thinning of the media and fragmentation of internal elastic lamina. The adventitia undergoes fibrosis and some inflammatory changes. These changes cause weakening in the arterial wall resulting in aneurysmal dilatation.

Clinical Effects

The clinical effects of atherosclerosis depend upon the size and type of arteries affected. In general, the clinical effects result from the following:

1. Slow luminal narrowing causing ischaemia and atrophy.
2. Sudden luminal occlusion causing infarction necrosis.
3. Propagation of plaque by formation of thrombi and emboli.
4. Formation of aneurysmal dilatation and eventual rupture.

Large arteries affected most often are the aorta, renal, mesenteric and carotids, whereas the medium- and small-sized arteries frequently involved are the coronaries, cerebrals and arteries of the lower limbs. Accordingly, the symptomatic atherosclerotic disease involves most often the heart, brain, kidneys, small intes-

tine and lower extremities (Fig. 24.5). The effects pertaining to these organs are described in relevant chapters later. Some of the important effects are listed below (Fig. 24.6):

- i) *Aorta*—Aneurysm formation, thrombosis and embolisation to other organs.
- ii) *Heart*—Myocardial infarction, ischaemic heart disease.
- iii) *Brain*—Chronic ischaemic brain damage, cerebral infarction.
- iv) *Small intestine*—Ischaemic bowel disease, infarction.
- v) *Lower extremities*—Intermittent claudication, gangrene.

ISCHAEMIC HEART DISEASE

Ischaemic heart disease (IHD) is defined as acute or chronic form of cardiac disability arising from imbalance between the myocardial supply and demand for oxygenated blood. Since narrowing or obstruction of the coronary arterial system is the most common cause of myocardial anoxia, the alternate term '*coronary artery disease (CAD)*' is used synonymously with IHD. IHD or CAD is the leading cause of death in most industrialised countries (about one-third of all deaths) and somewhat low incidence is observed in the developing countries. Men develop IHD earlier than women and death rates are also slightly higher for men than for women until the menopause.

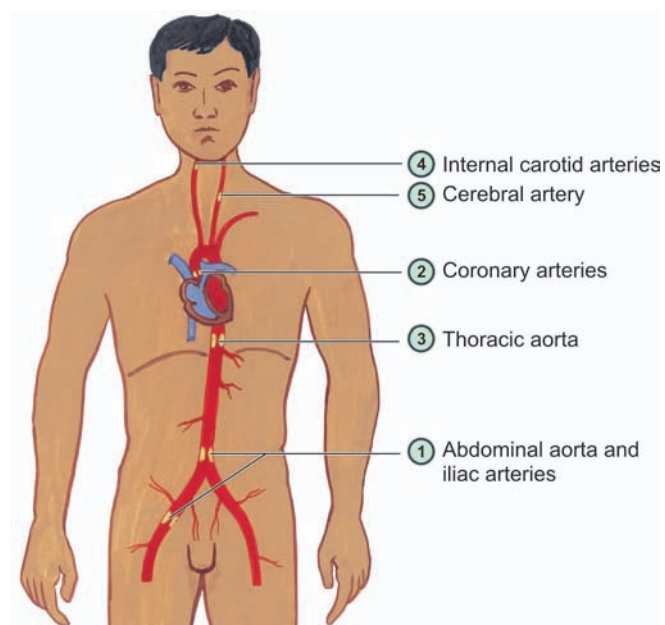


FIGURE 24.5

Major sites of atherosclerosis (serially numbered) in descending order of frequency.

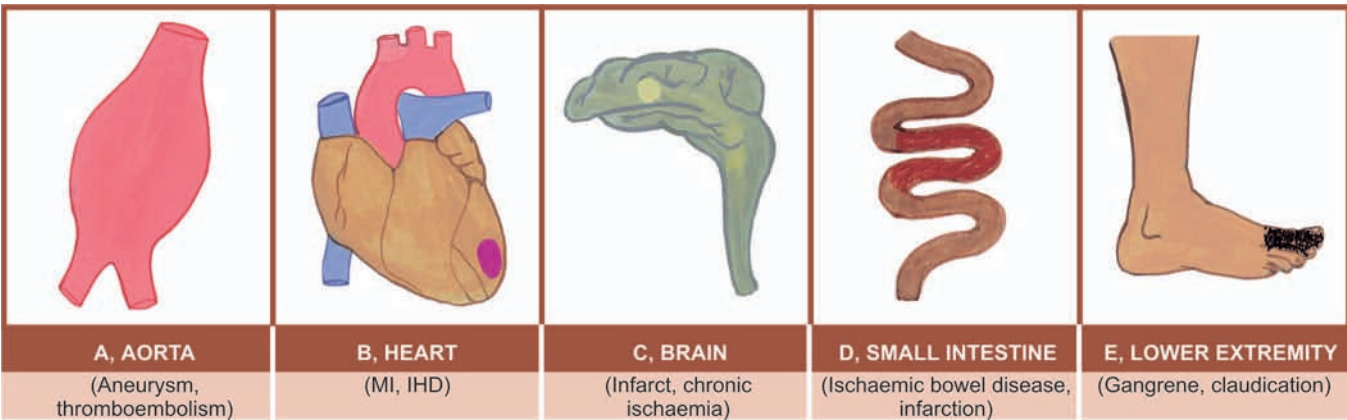


FIGURE 24.6

Major forms of symptomatic atherosclerotic disease.

ETIOPATHOGENESIS

IHD is invariably caused by disease affecting the coronary arteries, the most prevalent being atherosclerosis accounting for more than 90% cases, while other causes are responsible for less than 10% cases of IHD. Therefore, it is convenient to consider the etiology of IHD under three broad headings:

- coronary atherosclerosis;
- superadded changes in coronary atherosclerosis; and
- non-atherosclerotic causes.

I. Coronary Atherosclerosis

Coronary atherosclerosis resulting in 'fixed' obstruction is the major cause of IHD in more than 90% cases. The general aspects of atherosclerosis as regards its etiology, pathogenesis and the gross and microscopic features of atherosclerotic lesions have already been dealt with at length above. Here, a brief account of the pathology of lesions in *atherosclerotic coronary artery disease* is presented.

1. Distribution. Atherosclerotic lesions in coronary arteries are distributed in one or more of the three major coronary arterial trunks, the highest incidence being in the anterior descending branch of the left coronary, followed in decreasing frequency, by the right coronary artery and still less in circumflex branch of the left coronary. About one-third of cases have *single-vessel disease*, most often left anterior descending arterial involvement; another one-third have *two-vessel disease*, and the remainder have *three major vessel disease*.

2. Location. Almost all adults show atherosclerotic plaques scattered throughout the coronary arterial

system. However, significant stenotic lesions that may produce chronic myocardial ischaemia show more than 75% (three-fourth) reduction in the cross-sectional area of a coronary artery or its branch. The area of severest involvement is about 3 to 4 cm from the coronary ostia, more often at or near the bifurcation of the arteries, suggesting the role of haemodynamic forces in atherogenesis.

3. Fixed atherosclerotic plaques. The atherosclerotic plaques in the coronaries are more often eccentrically located bulging into the lumen from one side. Occasionally, there may be concentric thickening of the wall of the artery. Atherosclerosis produces gradual luminal narrowing that may eventually lead to 'fixed' coronary obstruction. The general features of atheromas of coronary arteries are similar to those affecting elsewhere in the body and may develop similar complications like calcification, coronary thrombosis, ulceration, haemorrhage, rupture and aneurysm formation.

II. Superadded Changes in Coronary Atherosclerosis

The attacks of *acute coronary syndromes*, namely acute myocardial infarction, unstable angina and sudden ischaemic death, are precipitated by certain changes superimposed on a pre-existing fixed coronary atheromatous plaque. These are as under:

1. Acute changes in chronic atheromatous plaque. Though chronic fixed obstructions are the most frequent cause of IHD, acute coronary episodes are often precipitated by sudden changes in chronic plaques such as plaque haemorrhage, fissuring, or ulceration that results in embolisation of atheromatous debris. Acute

plaque changes are brought about by factors such as sudden coronary artery spasm, tachycardia, intraplaque haemorrhage and hypercholesterolaemia.

2. Coronary artery thrombosis. Transmural acute myocardial infarction is often precipitated by partial or complete coronary thrombosis. The initiation of thrombus occurs due to surface ulceration of fixed chronic atheromatous plaque, ultimately causing complete luminal occlusion. The lipid core of plaque, in particular, is highly thrombogenic. Small fragments of thrombotic material are then dislodged which are embolised to terminal coronary branches and cause microinfarcts of the myocardium.

3. Local platelet aggregation and coronary artery spasm. Some cases of acute coronary episodes are caused by local aggregates of platelets on the atheromatous plaque, short of forming a thrombus. The aggregated platelets release vasospastic mediators such as thromboxane A₂ which may probably be responsible for coronary vasospasm in the already atherosclerotic vessel.

III. Non-atherosclerotic Causes

A number of other lesions may cause IHD in less than 10% of cases. These are as under:

1. Vasospasm. It has been possible to document vasospasm of one of the major coronary arterial trunks in patients with no significant atherosclerotic coronary narrowing which may cause angina or myocardial infarction.

2. Stenosis of coronary ostia. Coronary ostial narrowing may result from extension of syphilitic aortitis or from aortic atherosclerotic plaques encroaching on the opening.

3. Arteritis. Various types of inflammatory involvements of coronary arteries or small branches like in rheumatic arteritis, polyarteritis nodosa, thromboangiitis obliterans (Buerger's disease), Takayasu's disease, Kawasaki's disease, tuberculosis and other bacterial infections may contribute to myocardial damage.

4. Embolism. Rarely, emboli originating from elsewhere in the body may occlude the left coronary artery and its branches and produce IHD. The emboli may originate from bland thrombi, or from vegetations of bacterial endocarditis; rarely fat embolism and air embolism of coronary circulation may occur.

5. Thrombotic diseases. Another infrequent cause of coronary occlusion is from hypercoagulability of the

blood such as in shock, polycythaemia vera, sickle cell anaemia and thrombotic thrombocytopenic purpura.

6. Trauma. Contusion of a coronary artery from penetrating injuries may produce thrombotic occlusion.

7. Aneurysms. Extension of dissecting aneurysm of the aorta into the coronary artery may produce thrombotic coronary occlusion. Rarely, congenital, mycotic and syphilitic aneurysms may occur in coronary arteries and produce similar occlusive effects.

8. Compression. Compression of a coronary from outside by a primary or secondary tumour of the heart may result in coronary occlusion.

EFFECTS OF MYOCARDIAL ISCHAEMIA

Development of lesions in the coronaries is not always accompanied by cardiac disease. Depending upon the suddenness of onset, duration, degree, location and extent of the area affected by myocardial ischaemia, the range of changes and clinical features may vary from an asymptomatic state at one extreme to immediate mortality at another (Fig. 24.7):

A. *Asymptomatic state*

B. Angina pectoris (AP)

C. Acute myocardial infarction (AMI)

D. Chronic ischaemic heart disease (CIHD)/ Ischaemic cardiomyopathy/ Myocardial fibrosis

E. *Sudden cardiac death*

The term *acute coronary syndromes* include a triad of acute myocardial infarction, unstable angina and sudden cardiac death.

ACUTE MYOCARDIAL INFARCTION

Acute myocardial infarction (AMI) is the most important consequence of coronary artery disease. Many patients may die within the first few hours of the onset, while remainder suffer from effects of impaired cardiac function. A significant factor that may prevent or diminish the myocardial damage is the development of collateral circulation through anastomotic channels over a period of time. A regular and well-planned exercise programme is likely to encourage good collateral circulation.

INCIDENCE. In industrialised countries, MI accounts for 10-25% of all deaths. Due to the dominant etiologic role of coronary atherosclerosis in MI, the incidence of MI correlates well with the incidence of atherosclerosis in a geographic area.

Age. MI may virtually occur at all ages, though the incidence is higher in the elderly. About 5% of heart

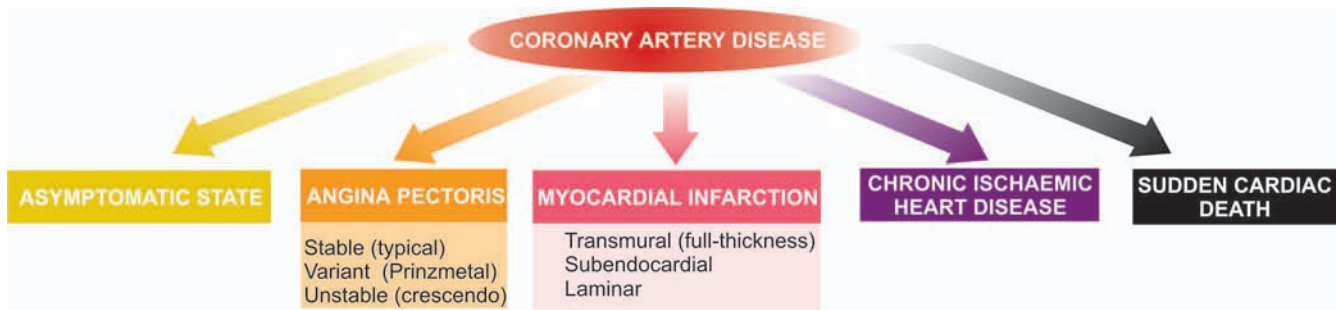


FIGURE 24.7

Spectrum of coronary ischaemic manifestations.

attacks occur in young people under the age of 40 years, particularly in those with major risk factors to develop atherosclerosis like hypertension, diabetes mellitus, cigarette smoking, familial hypercholesterolaemia etc.

Sex. Males throughout their life are at a significantly higher risk of developing MI as compared to females. Women during reproductive period have remarkably low incidence of MI, probably due to the protective influence of oestrogen. The use of oral contraceptives is associated with high risk of developing MI. After menopause, this sex difference gradually declines but the incidence of disease among women never reaches that among men of the same age.

ETIOPATHOGENESIS. The etiologic role of severe coronary atherosclerosis (more than 75% compromise of lumen) of one or more of the three major coronary arterial trunks in the pathogenesis of about 90% cases of acute MI is well documented by autopsy studies as well as by coronary angiographic studies. A few notable features in the etiology and pathogenesis of acute MI are considered below:

1. Mechanism of myocardial ischaemia. Myocardial ischaemia is brought about by one or more of the following mechanisms:

- Diminished coronary blood flow e.g. in coronary artery disease, shock.
- Increased myocardial demand e.g. in exercise, emotions.
- Hypertrophy of the heart without simultaneous increase of coronary blood flow e.g. in hypertension, valvular heart disease.

2. Role of platelets. Rupture of an atherosclerotic plaque exposes the subendothelial collagen to platelets which undergo aggregation, activation and release reaction. These events contribute to the build-up of the

platelet mass that may give rise to emboli or initiate thrombosis.

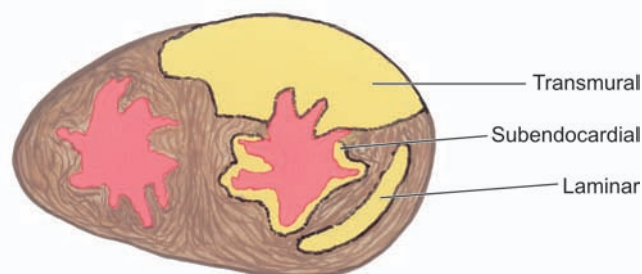
3. Complicated plaques. Two important complications in coronary atherosclerotic plaques which are frequently encountered are coronary thrombosis and haemorrhage:

- Superimposed coronary thrombosis* is seen in about half the cases of acute MI. Infusion of intracoronary fibrinolytics in the first few hours of development of acute MI in such cases restores blood flow in the blocked vessel in majority of cases.
- Intramural haemorrhage* is found in about one-third cases of acute MI. Haemorrhage and thrombosis may occur together in some cases.

4. Non-atherosclerotic causes. About 10% cases of acute MI are caused by non-atherosclerotic factors such as coronary vasospasm, arteritis, coronary ostial stenosis, embolism, thrombotic diseases, trauma and outside compression.

5. Transmural versus subendocardial infarcts. There are some differences in the pathogenesis of the *transmural infarcts* involving the full thickness of ventricular wall and the *subendocardial (laminar) infarcts* affecting the inner subendocardial one-third to half. These are as under (Table 24.3, Fig. 24.8):

- Transmural (full thickness) infarcts* are the most common type seen in 95% cases. Critical coronary narrowing (more than 75% compromised lumen) is of great significance in the causation of such infarcts. Atherosclerotic plaques with superimposed thrombosis and intramural haemorrhage are significant in about 90% cases, and non-atherosclerotic causes in the remaining 10% cases.
- Subendocardial (laminar) infarcts* have their genesis in reduced coronary perfusion due to coronary atherosclerosis but without critical stenosis (not necessarily

**FIGURE 24.8**

Diagrammatic representation of extent of myocardial infarction in the depth of myocardium.

75% compromised lumen), aortic stenosis or haemorrhagic shock. This is because subendocardial myocardium is normally least well perfused by coronaries and thus is more vulnerable to any reduction in the coronary flow. Superimposed coronary thrombosis is frequently encountered in these cases too, and hence the beneficial role of fibrinolytic treatment in such patients.

TYPES OF INFARCTS. Infarcts have been classified in a number of ways by the physicians and the pathologists:

1. According to the anatomic region of the left ventricle involved, they are called anterior, posterior (inferior), lateral, septal and circumferential, and their combinations like anterolateral, posterolateral (or inferolateral) and anteroseptal.
2. According to the degree of thickness of the ventricular wall involved, infarcts are of two types (Fig. 24.8):
 - i) Full-thickness or transmural, when they involve the entire thickness of the ventricular wall.
 - ii) Subendocardial or laminar, when they occupy the inner subendocardial half of the myocardium.
3. According to the age of infarcts, they are of two types:
 - i) Newly-formed infarcts are called acute, recent or fresh.
 - ii) Advanced infarcts are called old, healed or organised.

LOCATION OF INFARCTS. Infarcts are most frequently located in the left ventricle. Right ventricle is less susceptible to infarction due to its thin wall, having less metabolic requirements and is thus adequately nourished by the thebesian vessels. Atrial infarcts, whenever present, are more often in the right atrium, usually accompanying the infarct of the left ventricle. Left atrium is relatively protected from infarction because it is supplied by the oxygenated blood in the left atrial chamber.

The region of infarction depends upon the area of obstructed blood supply by one or more of the three coronary arterial trunks. Accordingly, there are three regions of myocardial infarction (Fig. 24.9):

1. *Stenosis of the left anterior descending coronary artery* is the most common (40-50%). The region of infarction is the anterior part of the left ventricle including the apex and the anterior two-thirds of the interventricular septum.
2. *Stenosis of the right coronary artery* is the next most frequent (30-40%). It involves the posterior part of the left ventricle and the posterior one-third of the interventricular septum.
3. *Stenosis of the left circumflex coronary artery* is seen least frequently (15-20%). Its area of involvement is the lateral wall of the left ventricle.

PATHOLOGIC CHANGES. The gross and microscopic changes in the myocardial infarction vary according to the age of the infarct and are therefore described sequentially (Table 24.4).

Grossly, most infarcts occur singly and vary in size from 4 to 10 cm. As explained above, they are found most often in the left ventricle. Less often, there are multifocal lesions. The transmural infarcts, which by definition involve the entire thickness of the ventricular wall, usually have a thin rim of preserved subendocardial myocardium which is perfused directly by the blood in the ventricular chamber. The subendocardial infarcts which affect the inner subendocardial half of the myocardium produce less well-defined gross changes than the transmural infarcts.

TABLE 24.3: Contrasting Features of Subendocardial and Transmural Infarcts.

FEATURE	TRANSMURAL INFARCT	SUBENDOCARDIAL INFARCT
1. Definition	Full-thickness, solid	Inner third to half, patchy
2. Frequency	Most frequent (95%)	Less frequent
3. Distribution	Specific area of coronary supply	Circumferential
4. Pathogenesis	> 75% coronary stenosis	Hypoperfusion of myocardium
5. Coronary thrombosis	Common	Rare
6. Epicarditis	Common	None

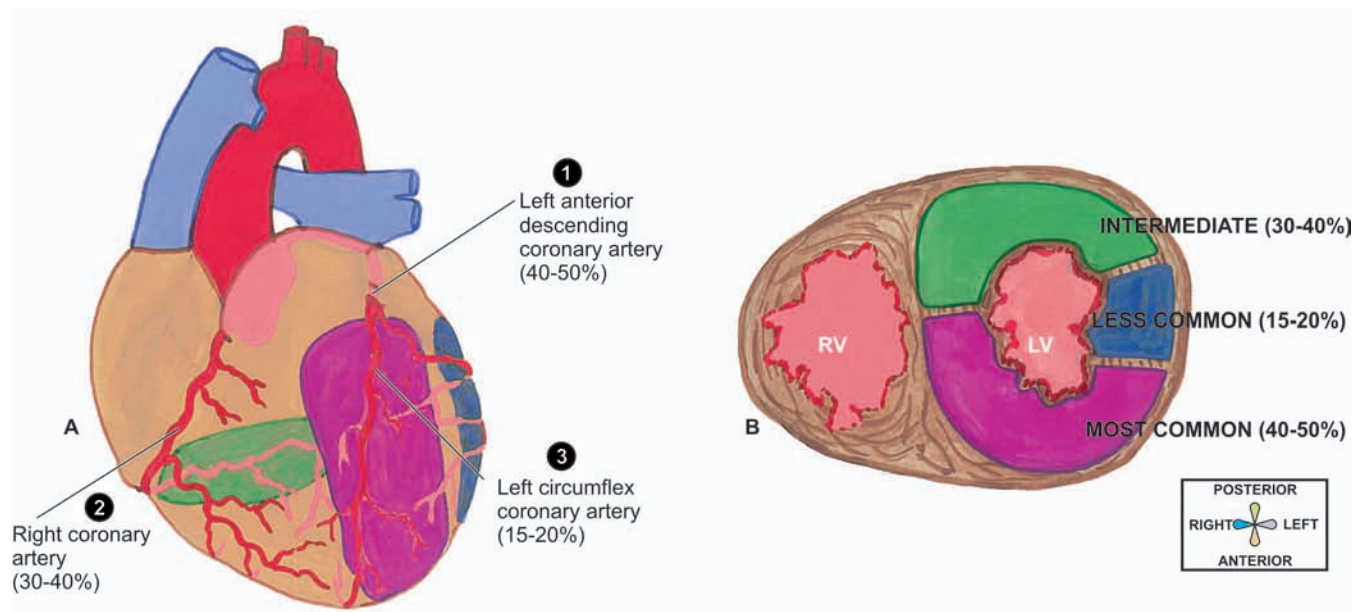


FIGURE 24.9

Common locations and the regions of involvement in myocardial infarction The figure shows region of myocardium affected by stenosis of three respective coronary trunks in descending order shown as: 1) left anterior descending coronary, 2) right coronary and 3) left circumflex coronary artery. A, As viewed from anterior surface. B, As viewed on transverse section at the apex of the heart.

TABLE 24.4: Sequential Pathologic Changes in Myocardial Infarction.

TIME	GROSS CHANGES	LIGHT MICROSCOPY
First week		
0-6 hours	No change or pale; TTC/ NBT test negative in infarcted area	No change; (?) stretching and waviness of fibres
6-12 hours	-do-	Coagulative necrosis begins; neutrophilic infiltration begins; oedema and haemorrhages present
24 hours	Cyanotic red-purple area of haemorrhage	Coagulative necrosis progresses; marginal neutrophilic infiltrate
48-72 hours	Pale, hyperaemic	Coagulative necrosis complete, neutrophilic infiltrate well developed
3-7th day	Hyperaemic border, centre yellow and soft	Neutrophils are necrosed and gradually disappear, beginning of resorption of necrosed fibres by macrophages, onset of fibrovascular response
Second week		
10th day	Red-purple periphery	Most of the necrosed muscle in a small infarct removed; fibrovascular reaction more prominent; pigmented macrophages, eosinophils, lymphocytes, plasma cells present
14th day	—	Necrosed muscle mostly removed; neutrophils disappear; fibrocollagenic tissue at the periphery
Third week	—	Necrosed muscle fibres from larger infarcts removed; more ingrowth of fibrocollagenic tissue
Fourth to sixth week	Thin, grey-white, hard, shrunken fibrous scar	Increased fibrocollagenic tissue, decreased vascularity; fewer pigmented macrophages, lymphocytes and plasma cells

The sequence of macroscopic changes in all myocardial infarcts is as under:

1. *In 6 to 12 hours old infarcts*, no striking gross changes are discernible except that the affected myocardium is slightly paler and drier than normal. However, the early infarcts (3 to 6 hours old) can be detected by histochemical staining for *dehydrogenases* on unfixed slice of the heart. This consists of immersing a slice of unfixed heart in the solution of triphenyltetrazolium chloride (TTC) which imparts red brown colour to the normal heart muscle, while the area of infarcted muscle fails to stain due to lack of dehydrogenases. Another stain for viability of cardiac muscle is nitroblue tetrazolium (NBT) dye which imparts blue colour to unaffected cardiac muscle while infarcted myocardium remains unstained.
2. *By about 24 hours*, the infarct develops cyanotic, red-purple, blotchy areas of haemorrhage due to stagnation of blood.
3. *During the next 48 to 72 hours*, the infarct develops a yellow border due to neutrophilic infiltration and thus becomes more well defined.
4. *In 3-7 days*, the infarct has hyperaemic border while the centre is yellow and soft.
5. *By 10 days*, the periphery of the infarct appears reddish-purple due to growth of granulation tissue. With the passage of time, further healing takes place; the necrotic muscle is resorbed and the infarct shrinks and becomes pale grey.
6. *By the end of 6 weeks*, the infarcted area is replaced by a thin, grey-white, hard, shrunken fibrous scar which is well developed in about 2 to 3 months. However, the time taken by an infarct to heal by fibrous scar may vary depending upon the size of the infarct and adequacy of collateral circulation.

Microscopically, the changes are similar in both transmural and subendocardial infarcts. As elsewhere in the body, myocardial ischaemia induces ischaemic coagulative necrosis of the myocardium which eventually heals by fibrosis. However, sequential light microscopic changes are observed as described below and illustrated in Fig. 24.10.

I. First week: The progression of changes takes place in the following way:

- i) *In the first 6 hours* after infarction, usually no detectable histologic change is observed in routine light microscopy. However, some investigators have described stretching and waviness of the myocardial fibres within one hour of the onset of ischaemia.

- ii) *After 6 hours*, there is appearance of some oedema fluid between the myocardial fibres. The muscle fibres at the margin of the infarct show vacuolar degeneration called myocytolysis.

- iii) *By 12 hours*, coagulative necrosis of the myocardial fibres sets in and neutrophils begin to appear at the margin of the infarct. Coagulative necrosis of fibres is characterised by loss of striations and intense eosinophilic, hyaline appearance and may show nuclear changes like karyolysis, pyknosis and karyorrhexis. Haemorrhages and oedema are present in the interstitium.

- iv) *During the first 24 hours*, coagulative necrosis progresses further as evidenced by shrunken eosinophilic cytoplasm and pyknosis of the nuclei. The neutrophilic infiltrate at the margins of the infarct is slight (Fig. 24.10,A).

- v) *During the first 48 to 72 hours*, coagulative necrosis is complete with loss of nuclei. The neutrophilic infiltrate is well developed and extends centrally into the interstitium (Fig. 24.10,B).

- vi) *In 3-7 days*, neutrophils are necrosed and gradually disappear. The process of resorption of necrosed muscle fibres by macrophages begins. Simultaneously, there is onset of proliferation of capillaries and fibroblasts from the margins of the infarct (Fig. 24.10,C).

2. Second week: The changes are as under:

- i) *By 10th day*, most of the necrosed muscle at the periphery of infarct is removed. The fibrovascular reaction at the margin of infarct is more prominent. Many pigmented macrophages containing yellow-brown lipofuscin (derived from breakdown of myocardial cells) and golden brown haemosiderin (derived from lysed erythrocytes in haemorrhagic areas) are seen. Also present are a few other inflammatory cells like eosinophils, lymphocytes and plasma cells.

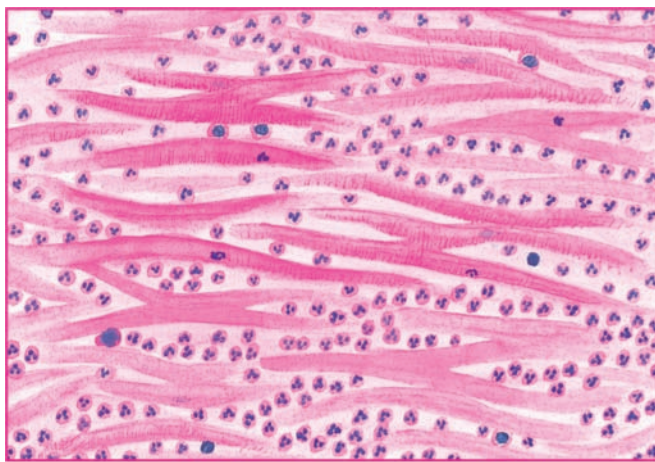
- ii) *By the end of the 2nd week*, most of the necrosed muscle in small infarcts is removed, neutrophils have almost disappeared, and newly laid collagen fibres replace the periphery of the infarct.

3. Third week: Necrosed muscle fibres from larger infarcts continue to be removed and replaced by ingrowth of newly formed collagen fibres. Pigmented macrophages as well as lymphocytes and plasma cells are prominent while eosinophils gradually disappear.

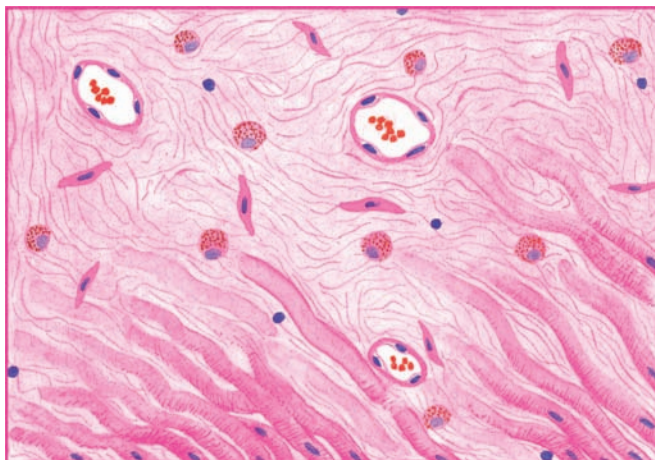
4. Fourth to sixth week: With further removal of necrotic tissue, there is increase in collagenous connective tissue, decreased vascularity and fewer pigmented macrophages, lymphocytes and plasma



A, CHANGES DURING THE FIRST 24 HOURS



B, CHANGES DURING THE FIRST 48-72 HOURS



C, CHANGES BY THE END OF FIRST WEEK

FIGURE 24.10

Sequence of light microscopic changes in myocardial infarction. (For details, consult the text).

cells. Thus, at the end of 6 weeks, a contracted fibro-collagenic scar with diminished vascularity is formed. The pigmented macrophages may persist for a long duration in the scar, sometimes for years.

A summary of the sequence of gross and microscopic changes in myocardial infarction of varying duration is presented in Table 24.4.

SALVAGE IN EARLY INFARCTS AND REPERFUSION INJURY. In vast majority of cases of AMI, occlusive coronary artery thrombosis has been demonstrated superimposed on fibrofatty plaque. The ischaemic injury to myocardium is reversible if perfusion is restored within the first 20-30 minutes of onset of infarction, failing which irreversible ischaemic necrosis of myocardium sets in. The salvage in early infarcts can be achieved by the following interventions:

1. Institution of *thrombolytic therapy* with thrombolytic agents such as streptokinase and tissue plasminogen activator.
2. *Percutaneous transluminal coronary angioplasty (PTCA)*.
3. *Coronary artery stenting*.
4. *Coronary artery bypass surgery*.

However, attempt at reperfusion is fraught with the risk of ischaemic reperfusion injury (Chapter 3). Further myonecrosis during reperfusion is possible due to rapid influx of calcium ions and generation of toxic oxygen free radicals.

PATHOLOGIC CHANGES. *Grossly*, the myocardial infarct following reperfusion injury appears *haemorrhagic* rather than pale.

Microscopically, myofibres show *contraction band necrosis* which are transverse and thick eosinophilic bands.

DIAGNOSIS. The diagnosis of acute MI is made on the observations of 3 types of features—clinical features, ECG changes, and serum enzyme determinations.

1. Clinical features. Typically, AMI has a sudden onset. The following clinical features usually characterise a case of AMI.

- i) *Pain*: Usually sudden, severe, crushing and prolonged, substernal or precordial in location, unrelieved by rest or nitroglycerin, often radiating to one or both the arms, neck and back.
- ii) *Indigestion*: Pain is often accompanied by epigastric or substernal discomfort interpreted as 'heartburn' with nausea and vomiting.
- iii) *Apprehension*: The patient is often terrified, restless and apprehensive due to great fear of death.

iv) *Shock*: Systolic blood pressure is below 80 mm Hg; lethargy, cold clammy limbs, peripheral cyanosis, weak pulse, tachycardia or bradycardia are often present.

v) *Oliguria*: Urine flow is usually less than 20 ml per hour.

vi) *Low grade fever*: Mild rise in temperature occurs within 24 hours and lasts upto one week, accompanied by leucocytosis and elevated ESR.

vii) *Acute pulmonary oedema*: Some cases develop severe pulmonary congestion due to left ventricular failure and develop suffocation, dyspnoea, orthopnoea and bubbling respiration.

2. ECG changes. The ECG changes are one of the most important parameters. Characteristic ECG changes include ST segment elevation, T wave inversion and appearance of wide deep Q waves (Fig. 24.11).

3. Serum cardiac markers. Certain proteins and enzymes are released into the blood from necrotic heart muscle after MI. Measurement of their levels in serum is helpful in making a diagnosis and plan management. Rapid assay of some more specific cardiac proteins is now available rendering the estimation of non-specific estimation of SGOT of historical importance only in current practice. Important myocardial markers in use nowadays are as under (Fig. 24.12):

i) *Creatine phosphokinase (CK) and CK-MB*: CK has three forms—

- CK-MM derived from skeletal muscle;
- CK-BB derived from brain and lungs; and
- CK-MB mainly from cardiac muscles and insignificant amount from extracardiac tissue.

Thus total CK estimation lacks specificity while elevation of CK-MB isoenzyme is considerably specific for myocardial damage. CK-MB has further 2 forms—CK-MB2 is the myocardial form while CK-MB1 is extracardiac form. A ratio of CK-MB2: CK-MB1 above 1.5 is highly sensitive for the diagnosis of acute MI after 4-6 hours of onset of myocardial ischaemia. CK-MB disappears from blood by 48 hours.

ii) *Lactic dehydrogenase (LDH)*. Total LDH estimation also lacks specificity since this enzyme is present in various tissues besides myocardium such as in skeletal muscle, kidneys, liver, lungs and red blood cells. However, like CK, LDH too has two isoforms of which LDH-1 is myocardial-specific. Estimation of ratio of LDH-1: LDH-2 above 1 is reasonably helpful in making a diagnosis. LDH levels begin to rise after 24 hours, reach peak in 3 to 6 days and return to normal in 14 days.

iii) *Cardiac-specific troponins (cTn)*: Immunoassay of cTn recently as a new cardiac serum marker has rendered LDH estimation obsolete. Troponins are contractile muscle proteins present in human cardiac and skeletal muscle but cardiac troponins are specific for myocardium. There are two types of cTn:

- cardiac troponin T (cTnT); and
- cardiac troponin I (cTnI).

Both cTnT and cTnI are not found in the blood normally, but after myocardial injury their levels rise very high around the same time when CK-MB is elevated (i.e. after 4-6 hours). Both troponin levels remain high for much longer duration; cTnI for 7-10 days and cTnT for 10-14 days.

iv) *Myoglobin*: Though myoglobin is the first cardiac marker to become elevated after myocardial infarction,

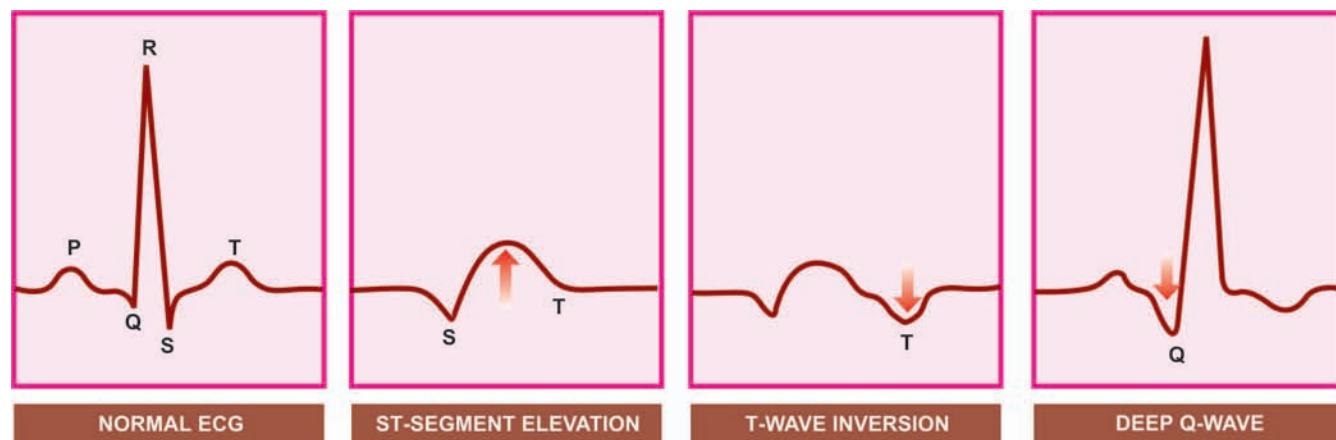


FIGURE 24.11

Some common ECG changes in acute myocardial infarction.

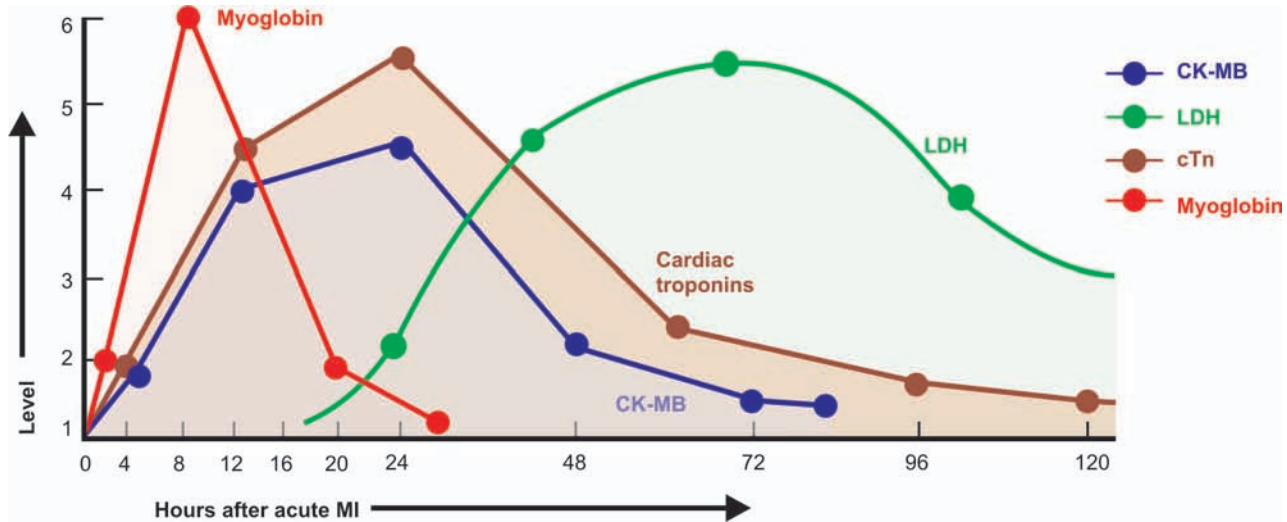


FIGURE 24.12

Time course of serum cardiac markers for the diagnosis of acute MI.

it lacks cardiac specificity and is excreted in the urine rapidly. Its levels, thus, return to normal within 24 hours of attack of acute MI.

COMPLICATIONS. Following an attack of acute MI, only 10-20% cases do not develop major complications and recover. The remainder 80-90% cases develop one or more major complications, some of which are fatal. The immediate mortality from acute MI (sudden cardiac death) is about 25%. The important complications which may develop following acute MI are as follows:

- 1. Arrhythmias.** Arrhythmias are the most common form of complications in acute MI. These occur due to ischaemic injury or irritation to the conduction system, resulting in abnormal rhythm. Other causes of arrhythmias include leakage of K^+ from ischaemic muscle cells and increased concentration of lactate and free fatty acids in the tissue fluid. Arrhythmias may be in the form of sinus tachycardia or sinus bradycardia, atrial fibrillation, premature systoles, and the most serious ventricular fibrillation responsible for many sudden cardiac deaths.

- 2. Congestive heart failure.** About half the patients with MI develop CHF which may be in the form of right ventricular failure, left ventricular failure or both. CHF is responsible for about 40% of deaths from acute MI. If the patient survives, healing may restore normal cardiac function but in some CHF may persist and require regular treatment later.

- 3. Cardiogenic shock.** About 10% of patients with acute MI develop cardiogenic shock characterised by hypotension with systolic blood pressure of 80 mmHg or less for many days. Shock may be accompanied by peripheral circulatory failure, oliguria and mental confusion.

- 4. Mural thrombosis and thromboembolism.** The incidence of thromboembolism from intracardiac thrombi and from thrombosis in the leg veins is 15-45% in cases of acute MI and is the major cause of death in 12% cases. Mural thrombosis in the heart develops due to involvement of the endocardium and subendocardium in the infarct and due to slowing of the heart rate. Mural thrombi often form thromboemboli. Another source of thromboemboli is the venous thrombosis in the leg veins due to prolonged bed rest. Thromboemboli from either source may cause occlusion of the pulmonary, renal, mesenteric, splenic, pancreatic or cerebral arteries and cause infarcts in these organs.

- 5. Rupture.** Rupture of heart occurs in upto 5% cases of acute MI causing death. Rupture occurs most often from the infarcted ventricular wall into the pericardial cavity causing haemopericardium and tamponade. Other sites of rupture are through interventricular septum and rupture of a papillary muscle in infarct of the left ventricle. Rupture at any of these sites occurs usually in the first week and is often fatal.

- 6. Cardiac aneurysm.** Another 5% of patients of MI develop aneurysm, often of the left ventricle. It occurs in healed infarcts through thin, fibrous, non-elastic scar

tissue. Cardiac aneurysms impair the function of the heart and are the common sites for mural thrombi. Rarely, calcification of the wall of aneurysm may occur.

7. Pericarditis. Sterile pericarditis appearing on about the second day is common over transmural infarcts. It is characterised by fibrinous pericarditis and may be associated with pericardial effusion. Often, it is of no functional significance and resolves spontaneously.

8. Postmyocardial infarction syndrome. About 3 to

4% of patients who suffered from acute MI develop postmyocardial infarction syndrome or *Dressler's syndrome* subsequently. It usually occurs 1 to 6 weeks after the attack of MI. It is characterised by pneumonitis. The symptoms are usually mild and disappear in a few weeks. The exact pathogenesis of this syndrome is not known. It may be due to autoimmune reaction as evidenced by circulating anti-heart antibodies in the serum of these patients. But these antibodies are also present in patients with MI who do not develop this syndrome.



RHEUMATIC HEART DISEASE

DEFINITION

Rheumatic fever (RF) is a systemic, post-streptococcal, non-suppurative inflammatory disease, principally affecting the heart, joints, central nervous system, skin and subcutaneous tissues. The chronic stage of RF involves all the layers of the heart (pancarditis) causing major cardiac sequelae referred to as rheumatic heart disease (RHD). In spite of its name suggesting an acute arthritis migrating from joint to joint, it is now well known that it is the heart rather than the joints which is first affected. William Boyd years ago gave the dictum '*rheumatism licks the joint, but bites the whole heart*'.

INCIDENCE

The disease appears most commonly in children between the age of 5 to 15 years when the streptococcal infection is most frequent and intense. Both the sexes are affected equally, though some investigators have noted a slight female preponderance.

The geographic distribution, incidence and severity of RF and RHD are generally related to the frequency and severity of streptococcal pharyngeal infection. The disease is seen more commonly in poor socioeconomic strata of the society living in damp and overcrowded places which promote interpersonal spread of the streptococcal infection. Its incidence has declined in the developed countries as a result of improved living conditions and use of antibiotics in streptococcal infection. But it is still common in the developing countries of the world like in India, Pakistan, some Arab countries, parts of Africa and South America. In India, RHD and RF continue to a major public health problem. In a multicentric survey in school-going children by the Indian Council of Medical Research, an incidence of 1 to 5.5 per 1000 children has been reported.

ETIOPATHOGENESIS

After a long controversy, the etiologic role of preceding throat infection with β -haemolytic streptococci of group A in RF is now generally accepted. However, the mechanism of lesions in the heart, joints and other tissues

is not by direct infection but by induction of hypersensitivity or autoimmunity. Thus, there are 2 types of evidences in the etiology and pathogenesis of RF and RHD: the *epidemiologic evidence* and the *immunologic evidence*.

A. EPIDEMIOLOGIC EVIDENCE. There is a body of clinical and epidemiological evidence to support the concept that RF occurs following infection of the throat and upper respiratory tract with β -haemolytic streptococci of Lancefield group A. These evidences are as under:

1. There is often a *history* of infection of the pharynx and upper respiratory tract with this microorganism about 2 to 3 weeks prior to the attack of RF. This period is usually the latent period required for sensitisation to the bacteria.
2. *Subsequent attacks* of streptococcal infection are generally associated with exacerbations of RF.
3. A higher incidence of RF has been observed after outbreaks and *epidemics* of streptococcal infection of throat in children from schools or in youngmen from training camps.
4. Administration of *antibiotics* leads to lowering of the incidence as well as severity of RF and its recurrences.
5. Cardiac lesions similar to those seen in RHD have been produced in experimental animals by *induction* of repeated infection with β -haemolytic streptococci of group A.
6. Patients with RF have *elevated titres* of antibodies to the antigens of β -haemolytic streptococci of group A such as antistreptolysin O (ASO) and S, antistreptokinase, antistreptohyaluronidase and anti- DNAase B.
7. *Socioeconomic factors* like poverty, poor nutrition, density of population, overcrowding in quarters for sleeping etc are associated with spread of infection, lack of proper medical attention, and hence higher incidence of RF.
8. The *geographic distribution* of the disease, as already pointed out, shows higher frequency and severity of the disease in the developing countries of the world where the living conditions are substandard and medical facilities are insufficient. Populations in these

regions develop recurrent throat infections which remain untreated and have higher incidence of RF.

9. The role of *climate* in the development of RF has been reported by some workers. The incidence of the disease is higher in subtropical and tropical regions with cold, damp climate near the rivers and water-ways which favour the spread of infection.

10. The *individual susceptibility* to RF and familial incidence have been reported. The factors contributing to proneness to develop RF include adverse social conditions, presence of streptococcal carrier at home and, as yet unclear role of hereditary defect.

Despite all these evidences, only a small proportion of patients with streptococcal pharyngeal infection develop RF—the attack rate is less than 3%. There is a suggestion that a *concomitant virus* enhances the effect of streptococci in individuals who develop RF.

B. IMMUNOLOGIC EVIDENCE. It has been observed that though throat of patients during acute RF contain streptococci, the clinical symptoms of RF appear after a delay of 2-3 weeks and the organisms can not be grown from the lesions in the target tissues. This has led to the concept that lesions are produced as a result of immune response by formation of autoantibodies against bacteria. A number of components of *Streptococcus* identify or cross-react with target human tissues in RHD i.e. cardiac muscle, valves, joints, skin, neurons etc. One such important component is *M-protein* identified as a surface protein of streptococcus which has various antigenic types, and hence corresponding antibodies in humans which target different tissues. The evidences in support are as under:

1. *Cell wall polysaccharide* of group A streptococcus forms antibodies which are reactive against cardiac valves. This is supported by observation of persistently elevated corresponding autoantibodies in patients who have cardiac valvular involvement than those without cardiac valve involvement.
2. *Hyaluronate capsule* of group A streptococcus is identical to human hyaluronate present in joint tissues and thus these tissues are the target of attack.
3. *Membrane antigens* of group A streptococcus react with sarcolemma of smooth and cardiac muscle, dermal fibroblasts and neurons of caudate nucleus.

PATHOLOGIC CHANGES

RF is generally regarded as an autoimmune focal inflammatory disorder of the connective tissues throughout the body. The *cardiac lesions* of RF in the

form of pancarditis, particularly the valvular lesions, are its major manifestations. However, supportive connective tissues at other sites like the synovial membrane, periarticular tissue, skin and subcutaneous tissue, arterial wall, lungs, pleura and the CNS are all affected (*extracardiac lesions*).

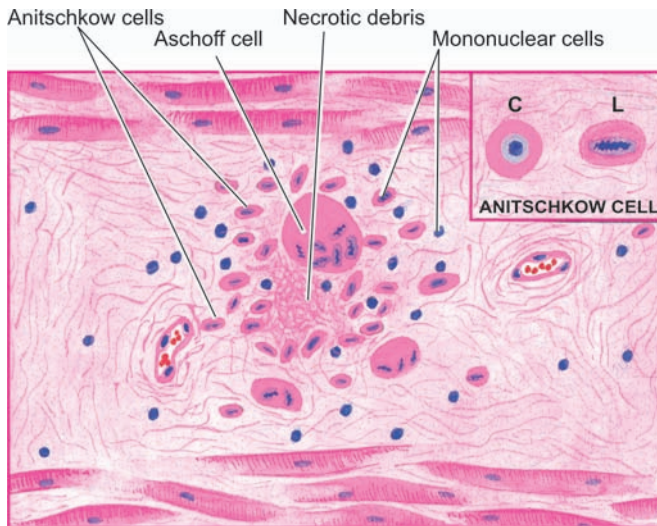
A. Cardiac Lesions

The cardiac manifestations of RF are in the form of focal inflammatory involvement of the interstitial tissue of all the three layers of the heart, the so-called *pancarditis*. The pathognomonic feature of pancarditis in RF is the presence of distinctive *Aschoff nodules* or *Aschoff bodies*.

THE ASCHOFF NODULES OR BODIES. The Aschoff nodules or the Aschoff bodies are spheroidal or fusiform distinct tiny structures, 1-2 mm in size, occurring in the interstitium of the heart in RF and may be visible to naked eye. They are especially found in the vicinity of small blood vessels in the myocardium and endocardium and occasionally in the pericardium and the adventitia of the proximal part of the aorta. Lesions similar to the Aschoff nodules may be found in the extracardiac tissues.

Evolution of fully-developed Aschoff bodies involves 3 stages all of which may be found in the same heart at different stages of development. These are as follows:

1. **Early (exudative or degenerative) stage.** The earliest sign of injury in the heart in RF is apparent by *about 4th week* of illness. Initially, there is oedema of the connective tissue and increase in acid mucopolysaccharide in the ground substance. This results in separation of the collagen fibres by accumulating ground substance. Eventually, the collagen fibres are fragmented and disintegrated and the affected focus takes the appearance and staining characteristics of fibrin. This change is referred to as *fibrinoid degeneration*.
2. **Intermediate (proliferative or granulomatous) stage.** It is this stage of the Aschoff body which is pathognomonic of rheumatic conditions (Fig. 25.1). This stage is apparent in 4th to 13th week of illness. The early stage of fibrinoid change is followed by proliferation of cells that includes infiltration by lymphocytes (mostly T cells), plasma cells, a few neutrophils and the characteristic *cardiac histiocytes* (*Anitschkow cells*) at the margin of the lesion. Cardiac histiocytes or Anitschkow cells are present in small numbers in normal heart but their number is increased in the Aschoff bodies; therefore they are

**FIGURE 25.1**

An Aschoff body (granulomatous stage) in the myocardium. *Inbox* shows Anitschkow cell in longitudinal section (L) with caterpillar-like serrated nuclear chromatin, while cross-section (C) shows owl-eye appearance of central chromatin mass and perinuclear halo.

not characteristic of RHD. These are large mononuclear cells having central round nuclei and contain moderate amount of amphophilic cytoplasm. The nuclei are vesicular and contain prominent central chromatin mass which in longitudinal section appears serrated or caterpillar-like, while in cross-section the chromatin mass appears as a small rounded body in the centre of the vesicular nucleus, just like an owl's eye (Fig. 25.1, *inbox*). Some of these modified cardiac histiocytes become multinucleate cells containing 1 to 4 nuclei and are called *Aschoff cells* and are pathognomonic of RHD.

3. Late (healing or fibrous) stage. The stage of healing by fibrosis of the Aschoff nodule occurs in about 12 to 16 weeks after the illness. The nodule becomes oval or fusiform in shape, about 200 μm wide and 600 μm long. The Anitschkow cells in the nodule become spindle-shaped with diminished cytoplasm and the nuclei stain solidly rather than showing vesicular character. These cells tend to be arranged in a palisaded manner. With passage of months and years, the Aschoff body becomes less cellular and the collagenous tissue is increased. Eventually, it is replaced by a small fibrocollagenous scar with little cellularity, frequently located perivascularly.

RHEUMATIC PANCARDITIS. Although all the three layers of the heart are affected in RF, the intensity of their involvement is variable.

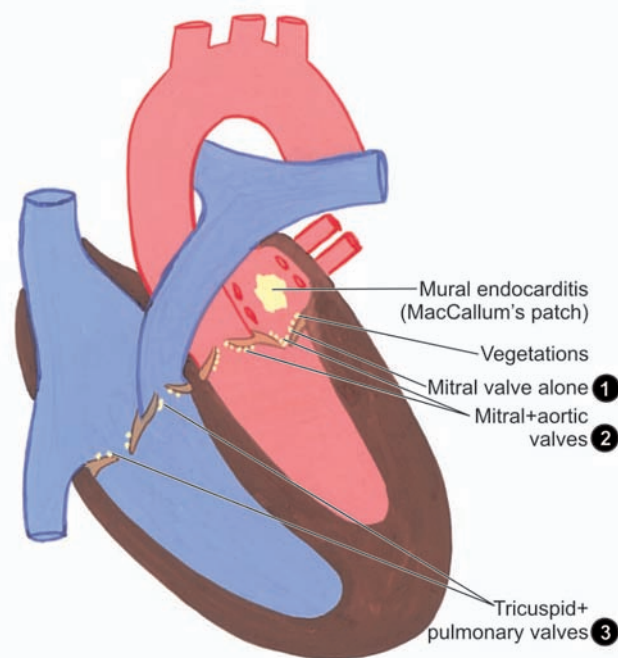
1. RHEUMATIC ENDOCARDITIS. Endocardial lesions of RF may involve the valvular and mural endocardium, causing *rheumatic valvulitis* and *mural endocarditis*, respectively. Rheumatic valvulitis is chiefly responsible for the major cardiac manifestations in chronic RHD.

RHEUMATIC VALVULITIS. *Grossly*, the valves in **acute RF** show thickening and loss of translucency of the valve leaflets or cusps. This is followed by the formation of characteristic, small (1 to 3 mm in diameter), multiple, warty *vegetations* or *verrucae*, chiefly along the line of closure of the leaflets and cusps. These tiny vegetations are almost continuous so that the free margin of the cusps or leaflets appears as a rough and irregular ridge. The vegetations in RF appear grey-brown, translucent and are firmly attached so that they are not likely to get detached to form emboli, unlike the friable vegetations of infective endocarditis (page 324).

Though all the four heart valves are affected, their frequency and severity of involvement varies: mitral valve alone being the most common site, followed in decreasing order of frequency, by combined mitral and aortic valve (Fig. 25.2). The tricuspid and pulmonary valves usually show infrequent and slight involvement. The higher incidence of vegetations on left side of the heart is possibly because of the greater mechanical stresses on the valves of the left heart, especially along the line of closure of the valve cusps (Fig. 25.3,A). The occurrence of vegetations on the atrial surfaces of the atrioventricular valves (mitral and tricuspid) and on the ventricular surface of the semilunar valves (aortic and pulmonary) further lends support to the role of mechanical pressure on the valves in the pathogenesis of vegetations.

The **chronic stage of RHD** is characterised by permanent deformity of one or more valves, especially the mitral (in 98% cases alone or along with other valves) and aortic. The approximate frequency of deformity of various valves is as under:

- Mitral alone=37% cases.
- Mitral+aortic=27% cases.
- Mitral+aortic+tricuspid=22% cases.
- Mitral+tricuspid=11% cases.
- Aortic alone=2%.
- Mitral+aortic+tricuspid+pulmonary=less than 1% cases.

**FIGURE 25.2**

Schematic representation of the anatomic regions of involvement and location of vegetations in rheumatic endocarditis (both valvular and mural). Serial numbers 1, 2 and 3 are denoted for the frequency of valvular involvement.

Thus, mitral valve is almost always involved in RHD. Gross appearance of chronic healed mitral valve in RHD is characteristically 'fish mouth' or 'button hole' stenosis. Mitral stenosis and insufficiency are commonly combined in chronic RHD; calcific aortic stenosis may also be found. These healed chronic valvular lesions in RHD occur due to diffuse fibrocollagenous thickening and calcification of the valve cusps or leaflets which cause adhesions between the lateral portions, especially in the region of the commissures. Thickening, shortening and fusion of the chordae tendineae further contribute to the chronic valvular lesions.

Microscopically, the inflammatory changes begin in the region of the valve rings (where the leaflets are attached to the fibrous annulus) and then extend throughout the entire leaflet, whereas vegetations are usually located on the free margin of the leaflets and cusps.

i) In the **early (acute) stage**, the histological changes are oedema of the valve leaflet, presence of increased number of capillaries and infiltration with lymphocytes, plasma cells, histiocytes with many Anitschkow cells and a few polymorphs. Occa-

sionally, Aschoff bodies with central foci of fibrinoid necrosis and surrounded by palisade of cardiac histiocytes are seen, but more often the cellular infiltration is diffuse in acute stage of RF. Vegetations present at the free margins of cusps appear as eosinophilic, tiny structures mainly consisting of fibrin with superimposed platelet-thrombi and do not contain bacteria (Fig. 25.3,B).

ii) In the **healed (chronic) stage**, the vegetations have undergone organisation. The valves show diffuse thickening as a result of fibrous tissue with hyalinisation, and often calcification. Vascularisation of the valve cusps may still be evident in the form of thick-walled blood vessels with narrowed lumina. Typical Aschoff bodies are rarely seen in the valves at this stage.

RHEUMATIC MURAL ENDOCARDITIS. Mural endocardium may also show features of rheumatic carditis though the changes are less conspicuous as compared to valvular changes.

Grossly, the lesions are seen most commonly as *MacCallum's patch* which is the region of endocardial surface in the posterior wall of the left atrium just above the posterior leaflet of the mitral valve. MacCallum's patch appears as a map-like area of thickened, roughened and wrinkled part of the endocardium (Fig. 25.2).

Microscopically, the appearance of MacCallum's patch is similar to that seen in rheumatic valvulitis. The affected area shows oedema, fibrinoid change in the collagen, and cellular infiltrate of lymphocytes, plasma cells and macrophages with many Anitschkow cells. Typical Aschoff bodies may sometimes be found.

2. RHEUMATIC MYOCARDITIS. **Grossly**, in the *early (acute) stage*, the myocardium, especially of the left ventricle, is soft and flabby. In the *intermediate stage*, the interstitial tissue of the myocardium shows small foci of necrosis. Later, tiny pale foci of the Aschoff bodies may be visible throughout the myocardium.

Microscopically, the most characteristic feature of rheumatic myocarditis is the presence of distinctive Aschoff bodies. These diagnostic nodules are scattered throughout the interstitial tissue of the myocardium and are most frequent in the interventricular septum, left ventricle and left atrium. Derangements of the conduction system may, thus, be present. The Aschoff bodies are best identified in the inter-

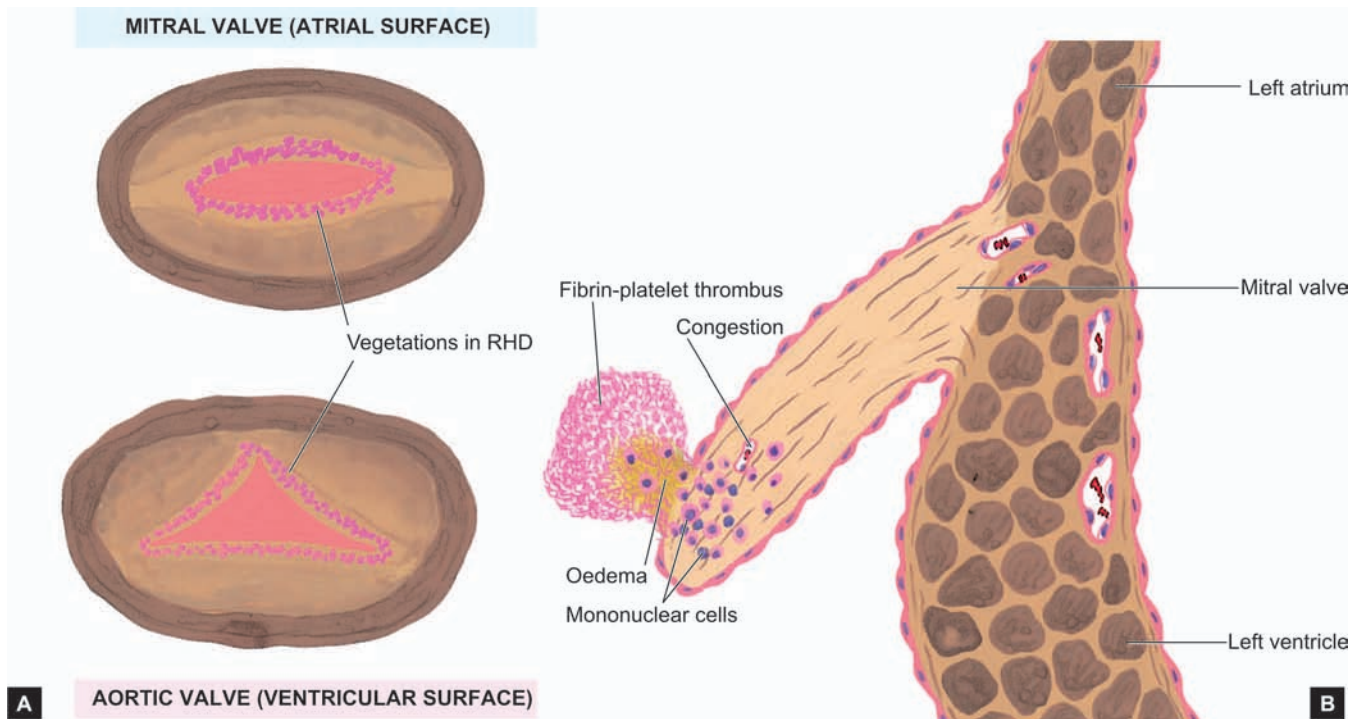


FIGURE 25.3

Rheumatic valvulitis. A, Location of vegetations on the valves of the left heart. The location of vegetations on mitral valve (above) is shown as viewed from the left atrium, while the vegetations on aortic valve (below) are shown as seen from the left ventricular surface. B, Microscopic structure of the rheumatic valvulitis and a vegetation on the cusp of mitral valve in sagittal section.

mediate stage when they appear as granulomas with central fibrinoid necrosis and are surrounded by palisade of Anitschkow cells and multinucleate Aschoff cells. There is infiltration by lymphocytes, plasma cells and some neutrophils. In the late stage, the Aschoff bodies are gradually replaced by small fibrous scars in the vicinity of blood vessels and the inflammatory infiltrate subsides. Presence of active Aschoff bodies along with old healed lesions is indicative of rheumatic activity.

3. RHEUMATIC PERICARDITIS. Inflammatory involvement of the pericardium commonly accompanies RHD.

Grossly, the usual finding is fibrinous pericarditis in which there is loss of normal shiny pericardial surface due to deposition of fibrin on its surface and accumulation of slight amount of fibrinous exudate in the pericardial sac. If the parietal pericardium is pulled off from the visceral pericardium, the two separated surfaces are shaggy due to thick fibrin covering them. This appearance is often likened to 'bread and butter appearance' i.e. resembling the buttered surfaces of

two slices in a sandwich when they are gently pulled apart. If fibrinous pericarditis fails to resolve and, instead, undergoes organisation, the two layers of the pericardium form fibrous adhesions resulting in chronic adhesive pericarditis.

Microscopically, fibrin is identified on the surfaces. The subserosal connective tissue is infiltrated by lymphocytes, plasma cells, histiocytes and a few neutrophils. Characteristic Aschoff bodies may be seen which later undergo organisation and fibrosis. Organisation of the exudate causes fibrous adhesions between the visceral and parietal surfaces of the pericardial sac and obliterates the pericardial cavity.

B. Extracardiac Lesions

Patients of the syndrome of acute rheumatism develop lesions in connective tissue elsewhere in the body, chiefly the joints, subcutaneous tissue, arteries, brain and lungs.

1. POLYARTHRITIS. Acute and painful inflammation of the synovial membranes of some of the joints, especially the larger joints of the limbs, is seen in about 90% cases of RF in adults and less often in children. As pain

and swelling subside in one joint, others tend to get involved, producing the characteristic '*migratory polyarthritis*' involving two or more joints at a time.

Microscopically, the changes are transitory. The synovial membrane and the periarticular connective tissue show hyperaemia, oedema, fibrinoid change and neutrophilic infiltration. Sometimes, focal lesions resembling Aschoff bodies are observed. A serous effusion into the joint cavity is commonly present.

2. SUBCUTANEOUS NODULES. The subcutaneous nodules of RF occur more often in children than in adult. These nodules are small (0.5 to 2 cm in diameter), spherical or ovoid and painless. They are attached to deeper structures like tendons, ligaments, fascia or periosteum and therefore often remain unnoticed by the patient. Characteristic locations are extensor surfaces of the wrists, elbows, ankles and knees.

Microscopically, the subcutaneous nodules of RF are representative of giant Aschoff bodies of the heart. They consist of 3 distinct zones: a central area with fibrinoid changes, surrounded by a zone of histiocytes and fibroblasts forming a palisade arrangement, and the outermost zone of connective tissue which is infiltrated by non-specific chronic inflammatory cells and proliferating blood vessels.

It may be mentioned here that histologically similar but clinically different subcutaneous lesions appear in rheumatoid arthritis; they are larger, painful and tender and persist for months to years.

3. ERYTHEMA MARGINATUM. This non-pruritic erythematous rash is characteristic of RF. The lesions occur mainly on the trunk and proximal parts of the extremities. The erythematous area develops central clearing and has slightly elevated red margins. The erythema is transient and migratory.

4. RHEUMATIC ARTERITIS. Arteritis in RF involves not only the coronary arteries and aorta but also occurs in arteries of various other organs such as renal, mesenteric and cerebral arteries. The lesions in the coronaries are seen mainly in the small intramyocardial branches.

Microscopically, the lesions may be like those of hypersensitivity angiitis, or sometimes may resemble polyarteritis nodosa. Occasionally, foci of fibrinoid necrosis or ill-formed Aschoff bodies may be present close to the vessel wall.

5. CHOREA MINOR. Chorea minor or Sydenham's chorea or Saint Vitus' dance is a delayed manifestation of RF as a result of involvement of the central nervous system. The condition is characterised by disordered and involuntary jerky movements of the trunk and the extremities accompanied by some degree of emotional instability. The condition occurs more often in younger age, particularly in girls.

Microscopically, the lesions are located in the cerebral hemispheres, brainstem and the basal ganglia. They consist of small haemorrhages, oedema and perivascular infiltration of lymphocytes. There may be endarteritis obliterans and thrombosis of cortical and meningeal vessels.

6. RHEUMATIC PNEUMONITIS AND PLEURITIS. Involvement of the lungs and pleura occurs rarely in RF. Pleuritis is often accompanied with serofibrinous pleural effusion but definite Aschoff bodies are not present. In rheumatic pneumonitis, the lungs are large, firm and rubbery.

Microscopically, the changes are oedema, capillary haemorrhages and focal areas of fibrinous exudate in the alveoli. Aschoff bodies are generally not found.

CLINICAL FEATURES

The first attack of acute RF generally appears 2 to 3 weeks after streptococcal pharyngitis, most often in children between the age of 5 to 15 years. With subsequent streptococcal pharyngitis, there is reactivation of the disease and similar clinical manifestations appear with each recurrent attack. The disease generally presents with migratory polyarthritis and fever. However, RF has widespread systemic involvement and no single specific laboratory diagnostic test is available. Therefore, for diagnosis, the following set of guidelines called *revised Jones' criteria* are followed:

A. Major criteria are:

1. Carditis
2. Polyarthritis
3. Chorea (Sydenham's chorea)
4. Erythema marginatum
5. Subcutaneous nodules

B. Minor criteria are:

1. Fever
2. Arthralgia
3. Previous history of RF

4. Laboratory findings of elevated ESR, raised C-reactive protein, and leucocytosis
5. ECG finding of prolonged PR interval.

C. Supportive evidence of preceding group A streptococcal infection: positive throat culture for group A streptococci, raised titres of streptococcal antibodies (antistreptolysin O and S, antistreptokinase, anti-streptohyaluronidase and anti DNAase B).

Clinical diagnosis of RF is made in a case with antecedent laboratory evidence of streptococcal throat infection in the presence of: any two of the major criteria, or occurrence of one major and two minor criteria.

If the heart is spared in a case of acute RF, the patient may have complete recovery without any sequelae. However, once the heart is involved, it is often associated with reactivation and recurrences of the disease. Myocarditis, in particular, is the most life-threatening due to involvement of the conduction system of the heart and results in serious arrhythmias. The long term sequelae or **stigmata** are the chronic valvular deformities, especially the mitral stenosis, as already explained on page 319. Initially, a state of compensation occurs, while later decompensation of the heart leads to full-blown cardiac failure. Currently, surgical replacement of the damaged valves can alter the clinical course of the disease.

The major **causes of death** in RHD are cardiac failure, bacterial endocarditis and embolism:

1. **Cardiac failure** is the most common cause of death from RHD. In young patients, cardiac failure occurs due to the chronic valvular deformities, while in older patients coronary artery disease may be superimposed on old RHD.
2. **Bacterial endocarditis** of both acute and subacute type may supervene due to inadequate use of antibiotics.
3. **Embolism** in RHD originates most commonly from mural thrombi in the left atrium and its appendages, in association with mitral stenosis. The organs most frequently affected are the brain, kidneys, spleen and lungs.
4. **Sudden death** may occur in RHD as a result of ball thrombus in the left atrium or due to acute coronary insufficiency in association with aortic stenosis.

BACTERIAL ENDOCARDITIS

DEFINITION. Bacterial endocarditis (BE) is serious infection of the valvular and mural endocardium caused by different forms of bacteria (other than tubercle bacilli and non-bacterial microorganisms) and is characterised

by typical infected and friable vegetations. Depending upon the severity of infection, BE is subdivided into 2 clinical forms:

1. Acute bacterial endocarditis (ABE) is the fulminant and destructive acute infection of the endocardium by highly virulent bacteria in a previously normal heart and almost invariably runs a rapidly fatal course in a period of 2-6 weeks.

2. Subacute bacterial endocarditis (SABE) or endocarditis lenta (*lenta* = slow) is caused by less virulent bacteria in a previously diseased heart and has a gradual downhill course in a period of 6 weeks to a few months and sometimes years.

Although classification of bacterial endocarditis into acute and subacute forms has been largely discarded because the clinical course is altered by antibiotic treatment, still a few important distinguishing features are worth mentioning (Table 25.1). However, characteristics of the vegetations in the two forms of BE are difficult to distinguish.

INCIDENCE. Introduction of antibiotic drugs has helped greatly in lowering the incidence of BE as compared with its incidence in the pre-antibiotic era. Though BE may occur at any age, most cases of ABE as well as SABE occur over 50 years of age. Males are affected more often than females.

ETIOLOGY. All cases of BE are caused by *infection with microorganisms* in patients having certain predisposing factors.

A. Infective agents. About 90% cases of BE are caused by streptococci and staphylococci.

■ In ABE, the most common causative organisms are virulent strains of staphylococci, chiefly *Staphylococcus*

TABLE 25.1: Distinguishing Features of Acute and Subacute Bacterial Endocarditis.

FEATURE	ACUTE	SUBACUTE
1. Duration	<6 weeks	>6 weeks
2. Most common organisms	<i>Staph. aureus</i> , β -streptococci	<i>Streptococcus viridans</i>
3. Virulence of organisms	Highly virulent	Less virulent
4. Previous condition of valves	Usually previously normal	Usually previously damaged
5. Lesion on valves	Invasive, destructive, suppurative	Usually not invasive or suppurative
6. Clinical features	Features of acute systemic infection	Splenomegaly, clubbing of fingers, petechiae

aureus. Others are pneumococci, gonococci, β -streptococci and enterococci.

■ In SABE, the commonest causative organisms are the streptococci with low virulence, predominantly *Streptococcus viridans*, which forms part of normal flora of the mouth and pharynx. Other less common etiologic agents include other strains of streptococci and staphylococci (e.g. *Streptococcus bovis* which is the normal inhabitant of gastrointestinal tract, *Streptococcus pneumoniae*, and *Staphylococcus epidermidis* which is a commensal of the skin), gram-negative enteric bacilli (e.g. *E. coli*, *Klebsiella*, *Pseudomonas* and *Salmonella*), pneumococci, gonococci and *Haemophilus influenzae*.

B. Predisposing factors. There are 3 main factors which predispose to the development of both forms of BE:

1. conditions initiating transient bacteraemia, septicaemia and pyaemia;
2. underlying heart disease; and
3. impaired host defenses.

1. *Bacteraemia, septicaemia and pyaemia:* Bacteria gain entrance to the bloodstream causing transient and clinically silent bacteraemia in a variety of day-to-day procedures as well as from other sources of infection. Some of the common examples are:

- i) Periodontal infections such as trauma from vigorous brushing of teeth, hard chewing, tooth extraction and other dental procedures.
- ii) Infections of the genitourinary tract such as in catheterisation, cystoscopy, obstetrical procedures including normal delivery and abortions.
- iii) Infections of gastrointestinal and biliary tract.
- iv) Surgery of the bowel, biliary tract and genitourinary tracts.
- v) Skin infections such as boils, carbuncles and abscesses.
- vi) Upper and lower respiratory tract infections including bacterial pneumonias.
- vii) Intravenous drug abuse.
- viii) Cardiac catheterisation and cardiac surgery for implantation of prosthetic valves.

2. *Underlying heart disease:* SABE occurs much more frequently in previously diseased heart valves, whereas the ABE is common in previously normal heart. Amongst the commonly associated underlying heart diseases are the following:

- i) Chronic rheumatic valvular disease in about 50% cases.
- ii) Congenital heart diseases in about 20% cases. These include VSD, subaortic stenosis, pulmonary stenosis, bicuspid aortic valve, coarctation of the aorta, and PDA.

iii) Other causes are syphilitic aortic valve disease, atherosclerotic valvular disease, floppy mitral valve, and prosthetic heart valves.

3. *Impaired host defenses:* All conditions in which there is depression of specific immunity, deficiency of complement and defective phagocytic function, predispose to BE. Following are some of the examples of such conditions:

- i) Impaired specific immunity in lymphomas.
- ii) Leukaemias.
- iii) Cytotoxic therapy for various forms of cancers and transplant patients.
- iv) Deficient functions of neutrophils and macrophages.

PATHOGENESIS. Bacteria on entering the bloodstream from any of the above-mentioned routes are implanted on the cardiac valves or mural endocardium. There are different hypotheses to explain the occurrence of bacterial implants on the valves:

1. The circulating bacteria are lodged much more frequently on *previously damaged valves* from diseases, chiefly RHD and congenital heart diseases, than on healthy valves.
2. Conditions producing *haemodynamic stress* on the valves are liable to cause damage to the endothelium, favouring the formation of platelet thrombi which get infected from circulating bacteria.
3. Another alternative hypothesis is the occurrence of *non-bacterial thrombotic endocarditis* from prolonged stress which is followed by bacterial contamination.

PATHOLOGIC CHANGES. The characteristic pathologic feature in both ABE and SABE is the presence of typical vegetations or verrucae on the valve cusps or leaflets, and less often, on mural endocardium.

Macroscopically, the lesions are found commonly on the valves of the left heart, most frequently on the mitral, followed in descending frequency, by the aortic, simultaneous involvement of both mitral and aortic valves, and quite rarely on the valves of the right heart. The vegetations in SABE are more often seen on previously diseased valves, whereas the vegetations of ABE are often found on previously normal valves. Like in RHD, the vegetations are often located on the atrial surface of atrioventricular valves and ventricular surface of the semilunar valves. They begin from the contact areas of the valve and may extend along the surface of the valves and on to the adjacent endocardium.

The *vegetations* of BE vary in size from a few millimeters to several centimeters, grey-tawny to

greenish, irregular, single or multiple, and typically *friable*. They may appear flat, filiform, fungating or polypoid. The vegetations in ABE tend to be bulkier and globular than those of SABE and are located more often on previously normal valves, may cause ulceration or perforation of the underlying valve leaflet, or may produce myocardial abscesses (Fig. 25.4,A).

Microscopically, the vegetations of BE consist of 3 zones (Fig. 25.4,B):

- i) The *outer layer or cap* consists of eosinophilic material composed of fibrin and platelets.
- ii) Underneath this layer is the *basophilic zone* containing colonies of bacteria. However, bacterial component of the vegetations may be lacking in treated cases.
- iii) The *deeper zone* consists of non-specific inflammatory reaction in the cusp itself, and in the case of SABE there may be evidence of repair.

In the acute fulminant form of the disease, the inflammatory cell infiltrate chiefly consists of neutrophils and is accompanied with tissue necrosis and abscesses in the valve rings and in the myocardium. In the subacute form, there is healing by granulation tissue,

mononuclear inflammatory cell infiltration and fibroblastic proliferation. Histological evidence of pre-existing valvular disease such as RHD may be present in SABE.

COMPLICATIONS AND SEQUELAE. Most cases of BE present with fever. The acute form of BE is characterised by high grade fever, chills, weakness and malaise while the subacute form of the disease has non-specific manifestations like slight fever, fatigue, loss of weight and flu-like symptoms. In the early stage, the lesions are confined to the heart, while subsequent progression of the disease leads to involvement of extracardiac organs. In general, severe complications develop early in ABE than in SABE. Complications and sequelae of BE are divided into cardiac and extracardiac (Fig. 25.5):

A. Cardiac complications. These include the following:

- i) Valvular stenosis or insufficiency
- ii) Perforation, rupture, and aneurysm of valve leaflets
- iii) Abscesses in the valve ring
- iv) Myocardial abscesses
- v) Suppurative pericarditis
- vi) Cardiac failure from one or more of the foregoing complications.

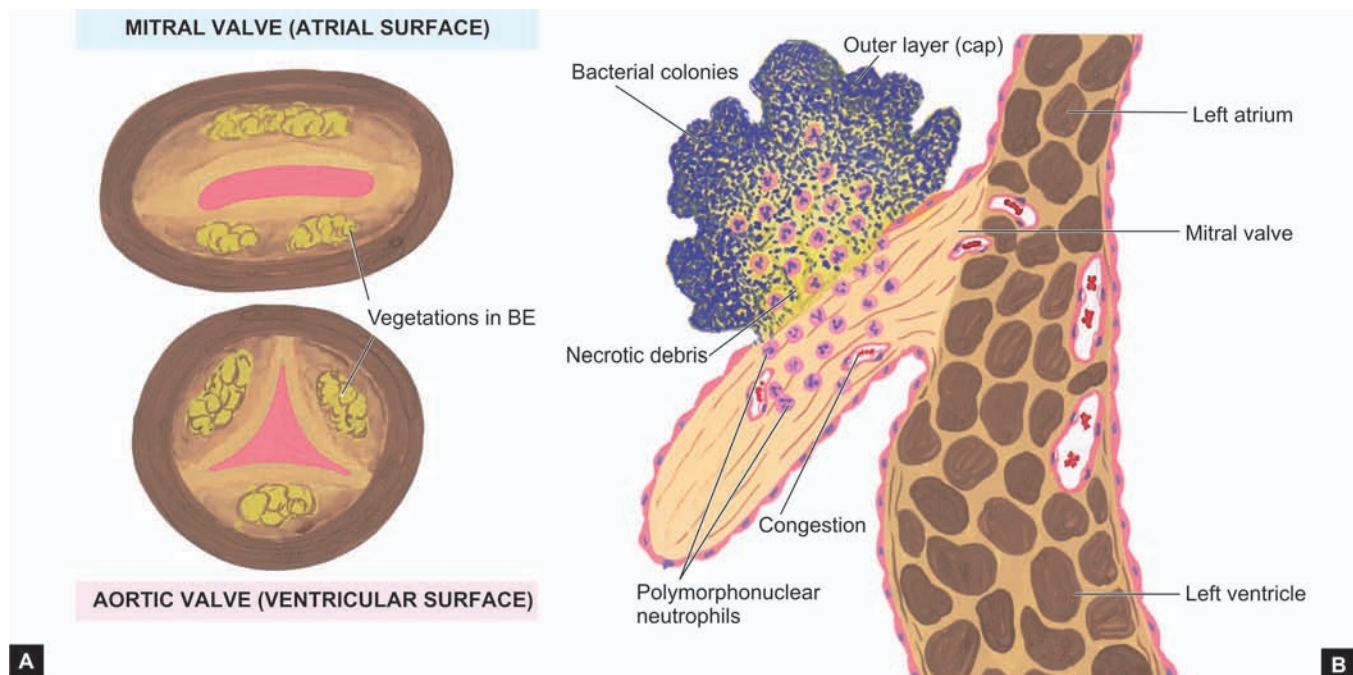
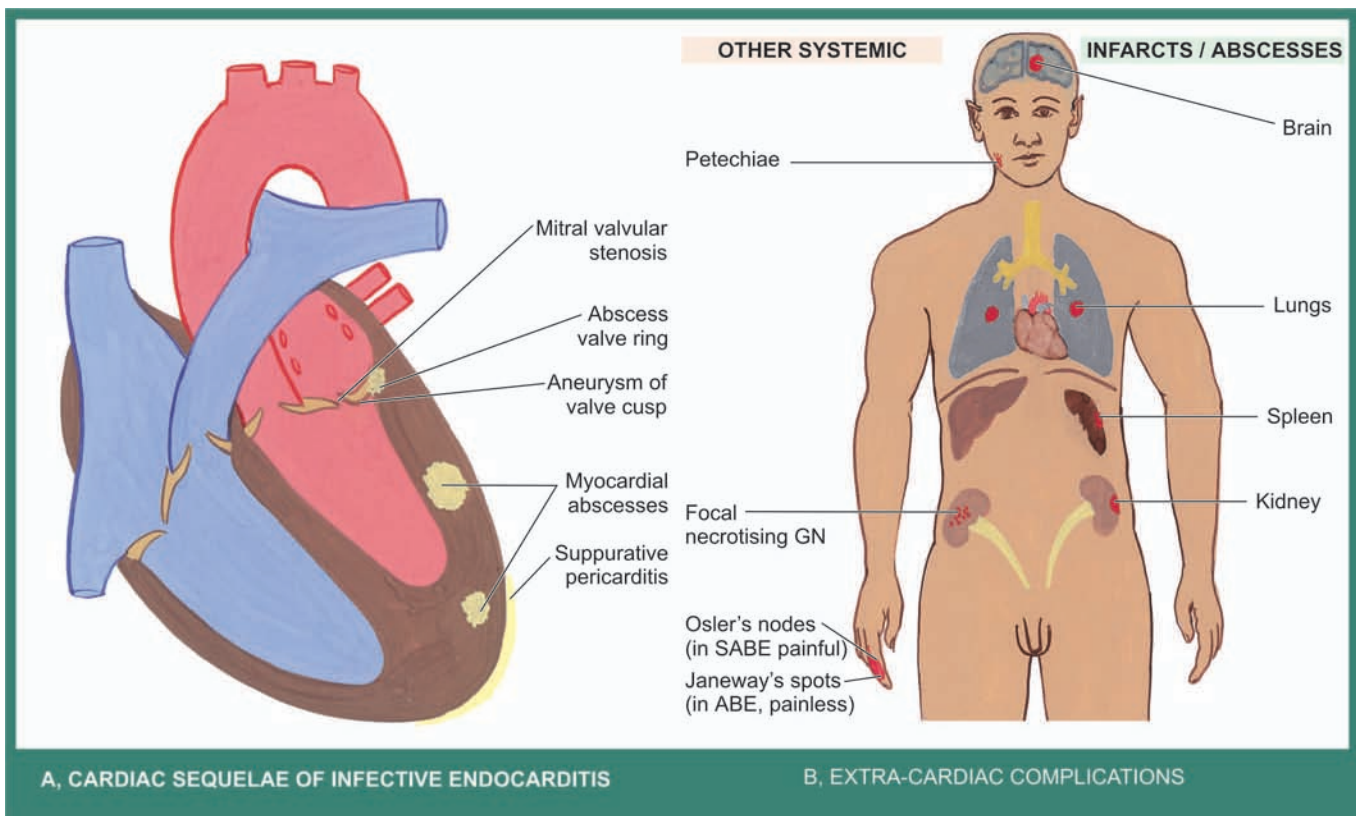


FIGURE 25.4

Bacterial endocarditis. A, Location of vegetations on the valves of the left heart. The vegetations are shown on the mitral valve (above) as viewed from the left atrium, while those on the aortic valve (below) are shown as seen from the left ventricle. B, Microscopic structure of a vegetation of BE on the surface of mitral valve in sagittal section.

**FIGURE 25.5**

Complications and sequelae of infective endocarditis.

B. Extracardiac complications. Since the vegetations in BE are typically friable, they tend to get dislodged due to rapid stream of blood and give rise to embolism which is responsible for very common and serious extra-cardiac complications. These are as under:

- Emboli originating from the *left side of the heart* and entering the systemic circulation affect organs like the spleen, kidneys, and brain causing infarcts, abscesses and mycotic aneurysms.
- Emboli arising from *right side of the heart* enter the pulmonary circulation and produce pulmonary abscesses.
- Petechiae* may be seen in the skin and conjunctiva due to either emboli or toxic damage to the capillaries.
- In SABE, there are painful, tender nodules on the finger tips of hands and feet called *Osler's nodes*, while

in ABE there is appearance of painless, non-tender subcutaneous maculopapular lesions on the pulp of the fingers called *Janeway's spots*. In either case, their origin is due to toxic or allergic inflammation of the vessel wall.

v) *Focal necrotising glomerulonephritis* is seen more commonly in SABE than in ABE. Occasionally diffuse glomerulonephritis may occur. Both these have their pathogenesis in circulating immune complexes (hypersensitivity phenomenon).

Treatment of BE with antibiotics in adequate dosage kills the bacteria but complications and sequelae of healed endocardial lesions may occur even after successful therapy. The *causes of death* are cardiac failure, persistent infection, embolism to vital organs, renal failure and rupture of mycotic aneurysm of cerebral arteries.



PNEUMONIAS

Pneumonia is defined as acute inflammation of the lung parenchyma distal to the terminal bronchioles which consist of the respiratory bronchiole, alveolar ducts, alveolar sacs and alveoli. The terms 'pneumonia' and 'pneumonitis' are often used synonymously for inflammation of the lungs, while 'consolidation' (meaning solidification) is the term used for macroscopic and radiologic appearance of the lungs in pneumonia.

PATHOGENESIS. The microorganisms gain entry into the lungs by one of the following four routes:

1. *Inhalation* of the microbes present in the air.
2. *Aspiration* of organisms from the nasopharynx or oropharynx.
3. *Haematogenous spread* from a distant focus of infection.
4. *Direct spread* from an adjoining site of infection.

The normal lung is free of bacteria because of the presence of a number of lung defense mechanisms at different levels such as nasopharyngeal filtering action, mucociliary action of the lower respiratory airways, the presence of phagocytosing alveolar macrophages and immunoglobulins. Failure of these defense mechanisms and presence of certain predisposing factors result in pneumonias. These conditions are as under:

1. **Altered consciousness.** The oropharyngeal contents may be aspirated in states causing unconsciousness e.g. in coma, cranial trauma, seizures, cerebrovascular accidents, drug overdose, alcoholism etc.
2. **Depressed cough and glottic reflexes.** Depression of effective cough may allow aspiration of gastric contents e.g. in old age, pain from trauma or thoraco-abdominal surgery, neuromuscular disease, weakness due to malnutrition, kyphoscoliosis, severe obstructive pulmonary diseases, endotracheal intubation and tracheostomy.
3. **Impaired mucociliary transport.** The normal protection offered by mucus-covered ciliated epithelium in the airways from the larynx to the terminal bronchioles is impaired or destroyed in many conditions favouring passage of bacteria into the lung parenchyma. These conditions are cigarette smoking, viral respiratory infections, immotile cilia syndrome, inhalation of hot or corrosive gases and old age.

4. **Impaired alveolar macrophage function.** Pneumonias may occur when alveolar macrophage function is impaired e.g. by cigarette smoke, hypoxia, starvation, anaemia, pulmonary oedema and viral respiratory infections.

5. **Endobronchial obstruction.** The effective clearance mechanism is interfered with in endobronchial obstruction from tumour, foreign body, cystic fibrosis and chronic bronchitis.

6. **Leucocyte dysfunctions.** Disorders of lymphocytes including congenital and acquired immunodeficiencies (e.g. AIDS, immunosuppressive therapy) and granulocyte abnormalities may predispose to pneumonia.

CLASSIFICATION. On the basis of the anatomic part of the lung parenchyma involved, pneumonias are traditionally classified into 3 main types:

1. Lobar pneumonia
2. Bronchopneumonia (or Lobular pneumonia)
3. Interstitial pneumonia

However, now that much is known about etiology and pathogenesis of pneumonias, current practice is to follow the etiologic classification (Table 26.1) which will be followed in the present discussion too.

A. BACTERIAL PNEUMONIA

Bacterial infection of the lung parenchyma is the most common cause of pneumonia or consolidation of one or both the lungs. Two types of acute bacterial pneumonias are distinguished—lobar pneumonia and

TABLE 26.1: Etiologic Classification of Pneumonias.

A. BACTERIAL PNEUMONIA

- I. Lobar pneumonia
- II. Bronchopneumonia (Lobular pneumonia)

B. VIRAL AND MYCOPLASMAL PNEUMONIA (PRIMARY ATYPICAL PNEUMONIA)**C. OTHER TYPES OF PNEUMONIAS**

- I. *Pneumocystis carinii* pneumonia
- II. *Legionella* pneumonia (Legionnaire's disease)
- III. Aspiration (inhalation) pneumonia
- IV. Hypostatic pneumonia
- V. Lipid pneumonia

broncho-(lobular-) pneumonia, each with distinct etiologic agent and morphologic changes. Another type distinguished by some workers separately is *confluent pneumonia* which combines the features of both lobar and bronchopneumonia and involves larger (confluent) areas in both the lungs irregularly, while others consider this as a variant of bronchopneumonia.

1. Lobar Pneumonia

Lobar pneumonia is an acute bacterial infection of a part of a lobe, the entire lobe, or even two lobes of one or both the lungs.

ETIOLOGY. Based on the etiologic microbial agent causing lobar pneumonia, the following types are described:

1. Pneumococcal pneumonia. More than 90% of all lobar pneumonias are caused by *Streptococcus pneumoniae*, a lancet-shaped diplococcus. Out of various types, type 3-*S. pneumoniae* causes particularly virulent form of lobar pneumonia. Pneumococcal pneumonia in majority of cases is community-acquired infection.

2. Staphylococcal pneumonia. *Staphylococcus aureus* causes pneumonia by haematogenous spread of infection from another focus or after viral infections.

3. Streptococcal pneumonia. β -haemolytic streptococci may rarely cause pneumonia such as in children after measles or influenza, in severely debilitated elderly patients and in diabetics.

4. Pneumonia by gram-negative aerobic bacteria. Less common causes of lobar pneumonia are gram-negative bacteria like *Haemophilus influenzae*, *Klebsiella pneumoniae* (*Friedlander's bacillus*), *Pseudomonas*, *Proteus* and *Escherichia coli*. *H. influenzae* commonly causes pneumonia in children below 3 years of age after a preceding viral infection.

PATHOLOGIC CHANGES. Laennec's original description divides lobar pneumonia into 4 sequential pathologic phases: *stage of congestion* (initial phase), *red hepatisation* (early consolidation), *grey hepatisation* (late consolidation) and *resolution*. However, these classic stages seen in untreated cases are found much less often nowadays due to administration of antibiotics and improved medical care.

In lobar pneumonia, as the name suggests, part of a lobe, a whole lobe, or two lobes are involved, sometimes bilaterally. The lower lobes are affected most commonly. The sequence of pathologic changes described below represents the inflammatory response of lungs in bacterial infection.

1. STAGE OF CONGESTION: INITIAL PHASE (Fig. 26.1,A). The initial phase represents the early

acute inflammatory response to bacterial infection and lasts for 1 to 2 days.

Grossly, the affected lobe is enlarged, heavy, dark red and congested. Cut surface exudes blood-stained frothy fluid.

Microscopically, typical features of acute inflammatory response to the organisms are seen. These are as under:

- i) Dilatation and congestion of the capillaries in the alveolar walls.
- ii) Pale eosinophilic oedema fluid in the air spaces.
- iii) A few red cells and neutrophils in the intra-alveolar fluid.
- iv) Numerous bacteria demonstrated in the alveolar fluid by Gram's staining.

2. RED HEPATISATION: EARLY CONSOLIDATION (Fig. 26.1,B). This phase lasts for 2 to 4 days. The term hepatisation in pneumonia refers to liver-like consistency of the affected lobe on cut section.

Grossly, the affected lobe is red, firm and consolidated. The cut surface of the involved lobe is airless, red-pink, dry, granular and has liver-like consistency. The stage of red hepatisation is accompanied by serofibrinous pleurisy.

Microscopically, the following features are observed:

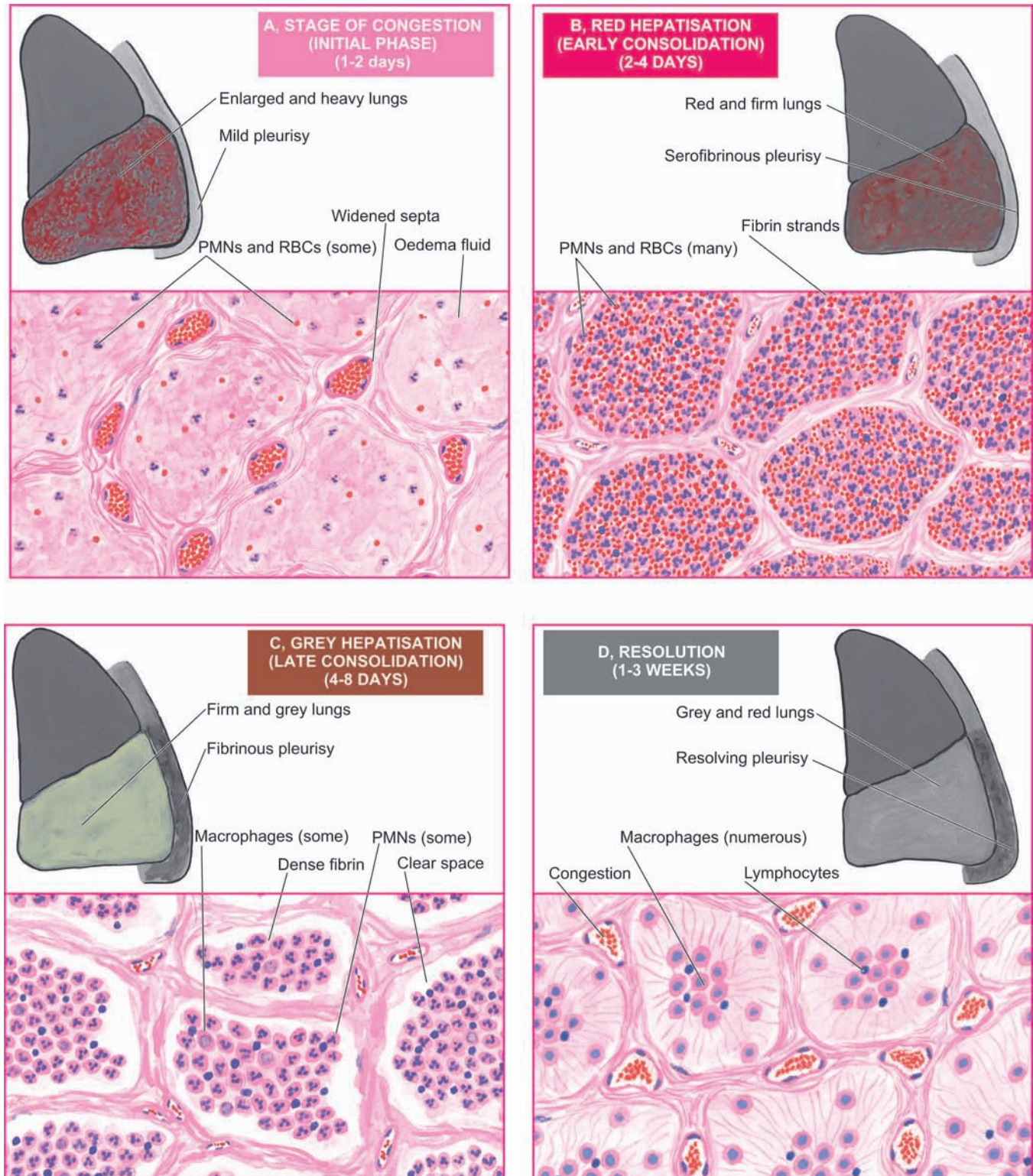
- i) The oedema fluid of the preceding stage is replaced by strands of fibrin.
- ii) There is marked cellular exudate of neutrophils and extravasation of red cells.
- iii) Many neutrophils show ingested bacteria.
- iv) The alveolar septa are less prominent than in the first stage due to cellular exudation.

3. GREY HEPATISATION: LATE CONSOLIDATION (Fig. 26.1,C). This phase lasts for 4 to 8 days.

Grossly, the affected lobe is firm and heavy. The cut surface is dry, granular and grey in appearance with liver-like consistency. The change in colour from red to grey begins at the hilum and spreads towards the periphery. Fibrinous pleurisy is prominent.

Microscopically, the following changes are present:

- i) The fibrin strands are dense and more numerous.
- ii) The cellular exudate of neutrophils is reduced due to disintegration of many inflammatory cells as evidenced by their pyknotic nuclei. The red cells are also fewer. The macrophages begin to appear in the exudate.
- iii) The cellular exudate is often separated from the septal walls by a thin clear space.
- iv) The organisms are less numerous and appear as degenerated forms.

**FIGURE 26.1**

The four stages of lobar pneumonia, showing correlation of gross appearance of the lung with microscopic appearance in each stage. For details consult the text.

4. RESOLUTION: (Fig. 26.1,D). This stage begins by 8th to 9th day if no chemotherapy is administered and is completed in 1 to 3 weeks. However, antibiotic therapy induces resolution on about 3rd day. Resolution proceeds in a progressive manner.

Grossly, the previously solid fibrinous constituent is liquefied by enzymatic action, eventually restoring the normal aeration in the affected lobe. The process of softening begins centrally and spreads to the periphery. The cut surface is grey-red or dirty brown and frothy, yellow, creamy fluid can be expressed on pressing. The pleural reaction may also show resolution but may undergo organisation leading to fibrous obliteration of pleural cavity.

Microscopically, the following features are noted:

- i) Macrophages are the predominant cells in the alveolar spaces, while neutrophils diminish in number. Many of the macrophages contain engulfed neutrophils and debris.
- ii) Granular and fragmented strands of fibrin in the alveolar spaces are seen due to progressive enzymatic digestion.
- iii) Alveolar capillaries are engorged.
- iv) There is progressive removal of fluid content as well as cellular exudate from the air spaces, partly by expectoration but mainly by lymphatics, resulting in restoration of normal lung parenchyma with aeration.

COMPLICATIONS. Since the advent of antibiotics, serious complications of lobar pneumonia are uncommon. However, they may develop in neglected cases and in patients with impaired immunologic defenses. These are as under:

- 1. Organisation.** In about 3% of cases, resolution of the exudate does not occur but instead it is organised. There is ingrowth of fibroblasts from the alveolar septa resulting in fibrosed, tough, airless leathery lung tissue. This type of post-pneumonic fibrosis is called *carnification*.
- 2. Pleural effusion.** About 5% of treated cases of lobar pneumonia develop inflammation of the pleura with effusion. The pleural effusion usually resolves but sometimes may undergo organisation with fibrous adhesions between visceral and parietal pleura.
- 3. Empyema.** Less than 1% of treated cases of lobar pneumonia develop encysted pus in the pleural cavity termed empyema.
- 4. Lung abscess.** A rare complication of lobar pneumonia is formation of lung abscess, especially when there is secondary infection by other organisms.

5. Metastatic infection. Occasionally, infection in the lungs and pleural cavity in lobar pneumonia may extend into the pericardium and the heart causing purulent pericarditis, bacterial endocarditis and myocarditis. Other forms of metastatic infection encountered rarely in lobar pneumonias are otitis media, mastoiditis, meningitis, brain abscess and purulent arthritis.

CLINICAL FEATURES. Classically, the onset of lobar pneumonia is sudden. The major symptoms are: shaking chills, fever, malaise with pleuritic chest pain, dyspnoea and cough with expectoration which may be mucoid, purulent or even bloody. The common physical findings are fever, tachycardia, and tachypnoea, and sometimes cyanosis if the patient is severely hypoxaemic. There is generally a marked neutrophilic leucocytosis. Blood cultures are positive in about 30% of cases. Chest radiograph may reveal consolidation. The culture of the organisms in the sputum and antibiotic sensitivity are most significant investigations for institution of specific antibiotics. The response to antibiotics is usually rapid with clinical improvement in 48 to 72 hours after the initiation of antibiotics.

II. Bronchopneumonia (Lobular Pneumonia)

Bronchopneumonia or lobular pneumonia is infection of the terminal bronchioles that extends into the surrounding alveoli resulting in patchy consolidation of the lung. The condition is particularly frequent at extremes of life (i.e. in infancy and old age), as a terminal event in chronic debilitating diseases and as a secondary infection following viral respiratory infections such as influenza, measles etc.

ETIOLOGY. The common organisms responsible for bronchopneumonia are staphylococci, streptococci, pneumococci, *Klebsiella pneumoniae*, *Haemophilus influenzae*, and gram-negative bacilli like *Pseudomonas* and coliform bacteria.

PATHOLOGIC CHANGES. **Grossly**, bronchopneumonia is identified by patchy areas of red or grey consolidation affecting one or more lobes, frequently found bilaterally and more often involving the lower zones of the lungs due to gravitation of the secretions (Fig. 26.2). On cut surface, these patchy consolidated lesions are dry, granular, firm, red or grey in colour, 3 to 4 cm in diameter, slightly elevated over the surface and are often centred around a bronchiole. These patchy areas are best picked up by passing the fingertips on the cut surface.

Microscopically, the following features are observed (Fig. 26.3) (COLOUR PLATE IV: CL 13):

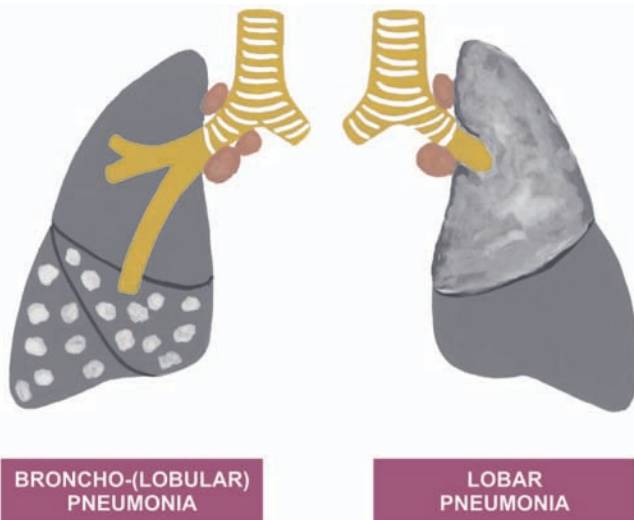


FIGURE 26.2

Gross appearance of bronchopneumonia contrasted with that of lobar pneumonia.

- i) Acute bronchiolitis.
- ii) Suppurative exudate, consisting chiefly of neutrophils, in the peribronchiolar alveoli.
- iii) Thickening of the alveolar septa by congested capillaries and leucocytic infiltration.
- iv) Less involved alveoli contain oedema fluid.

COMPLICATIONS. The complications of lobar pneumonia may occur in bronchopneumonia as well. However, complete resolution of bronchopneumonia is uncommon. There is generally some degree of destruction of the bronchioles resulting in foci of bronchiolar fibrosis that may eventually cause bronchiectasis.

CLINICAL FEATURES. The patients of bronchopneumonia are generally infants or elderly individuals. There may be history of preceding bed-ridden illness, chronic debility, aspiration of gastric contents or upper respiratory infection. For initial 2 to 3 days, there are features of acute bronchitis but subsequently signs and symptoms similar to those of lobar pneumonia appear. Blood examination usually shows a neutrophilic leucocytosis. Chest radiograph shows mottled, focal opacities in both the lungs, chiefly in the lower zones.

The salient features of the two main types of bacterial pneumonias are contrasted in Table 26.2.

B. VIRAL AND MYCOPLASMA PNEUMONIA (PRIMARY ATYPICAL PNEUMONIA)

Viral and mycoplasmal pneumonia is characterised by patchy inflammatory changes, largely confined to

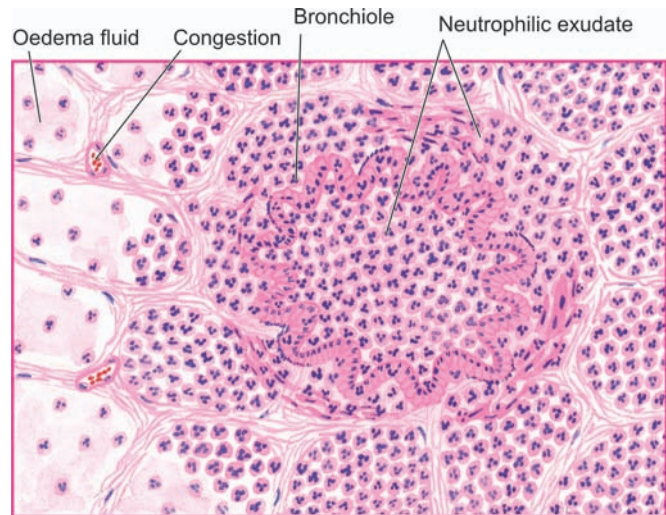


FIGURE 26.3

Microscopic appearance of bronchopneumonia. The bronchioles as well as the adjacent alveoli are filled with exudate consisting chiefly of neutrophils. The alveolar septa are thickened due to congested capillaries and neutrophilic infiltrate.

interstitial tissue of the lungs, without any alveolar exudate. Other terms used for these respiratory tract infections are *interstitial pneumonitis*, reflecting the interstitial location of the inflammation, and *primary atypical pneumonia*, atypicality being the absence of alveolar exudate commonly present in other pneumonias. Interstitial pneumonitis may occur in all ages. Most of the cases are mild and transient; exceptionally it may be severe and fulminant.

ETIOLOGY. Interstitial pneumonitis is caused by a wide variety of agents, the most common being *respiratory syncytial virus* (RSV). Others are *Mycoplasma pneumoniae* and many viruses such as influenza and parainfluenza viruses, adenoviruses, rhinoviruses, coxsackieviruses and cytomegaloviruses (CMV). Occasionally, psittacosis (*Chlamydia*) and Q fever (*Coxiella*) are associated with interstitial pneumonitis.

Infections of the respiratory tract with these organisms are quite common. In most cases, the infection remains confined to the upper respiratory tract presenting as common cold. Occasionally, it may extend lower down to involve the interstitium of the lungs. The circumstances favouring such extension of infection are malnutrition, chronic debilitating diseases and alcoholism.

PATHOLOGIC CHANGES. Irrespective of the etiologic agent, the pathologic changes are similar in all cases.

TABLE 26.2: Contrasting Features of Lobar Pneumonia and Bronchopneumonia.

FEATURE	LOBAR PNEUMONIA	BRONCHOPNEUMONIA
1. <i>Definition</i>	Acute bacterial infection of a part of a lobe of one or both lungs, or the entire lobe/s	Acute bacterial infection of the terminal bronchioles extending in to adjoining alveoli
2. <i>Age group</i>	More common in adults	Commoner at extremes of age—infants and old age
3. <i>Predisposing factors</i>	More often affects healthy individuals	Preexisting diseases e.g. chronic debility, terminal illness, flu, measles
4. <i>Common etiologic agents</i>	Pneumococci, <i>Klebsiella pneumoniae</i> , staphylococci, streptococci	Staphylococci, streptococci, <i>Pseudomonas</i> , <i>Haemophilus influenzae</i>
5. <i>Pathologic features</i>	Typical case passes through stages of congestion (1-2 days), early (2-4 days) and late consolidation (4-8 days), followed by resolution (1-3 weeks)	Patchy consolidation with central granularity, alveolar exudation, thickened septa
6. <i>Investigations</i>	Neutrophilic leucocytosis, positive blood culture, X-ray shows consolidation	Neutrophilic leucocytosis, positive blood culture, X-ray shows mottled focal opacities
7. <i>Prognosis</i>	Better response to treatment, resolution common, prognosis good	Response to treatment variable, organisation may occur, prognosis poor
8. <i>Complications</i>	Less common; pleural effusion, empyema, lung abscess, organisation	Brochiectasis may occur; other complications same as for lobar pneumonia

Grossly, depending upon the severity of infection, the involvement may be patchy to massive and widespread consolidation of one or both the lungs. The lungs are heavy, congested and subcrepitant. Sectioned surface of the lung exudes small amount of frothy or bloody fluid. The pleural reaction is usually infrequent and mild.

Microscopically, hallmark of viral pneumonias is the interstitial nature of the inflammatory reaction. The microscopic features are as under (Fig. 26.4):

i) Interstitial inflammation: There is thickening of alveolar walls due to congestion, oedema and mononuclear inflammatory infiltrate comprised by lymphocytes, macrophages and some plasma cells.

ii) Necrotising bronchiolitis: This is characterised by foci of necrosis of the bronchiolar epithelium, inspissated secretions in the lumina and mononuclear infiltrate in the walls and lumina.

iii) Reactive changes: The lining epithelial cells of the bronchioles and alveoli proliferate in the presence of virus and may form multinucleate giant cells and syncytia in the bronchiolar and alveolar walls. Occasionally, viral inclusions (intranuclear and/or intracytoplasmic) are found, especially in pneumonitis caused by CMV.

vi) Alveolar changes: In severe cases, the alveolar lumina may contain oedema fluid, fibrin, scanty

inflammatory exudate and coating of alveolar walls by pink, hyaline membrane similar to the one seen in respiratory distress syndrome. Alveolar changes are prominent if bacterial infection supervenes.

COMPLICATIONS. The major complication of interstitial pneumonitis is superimposed bacterial

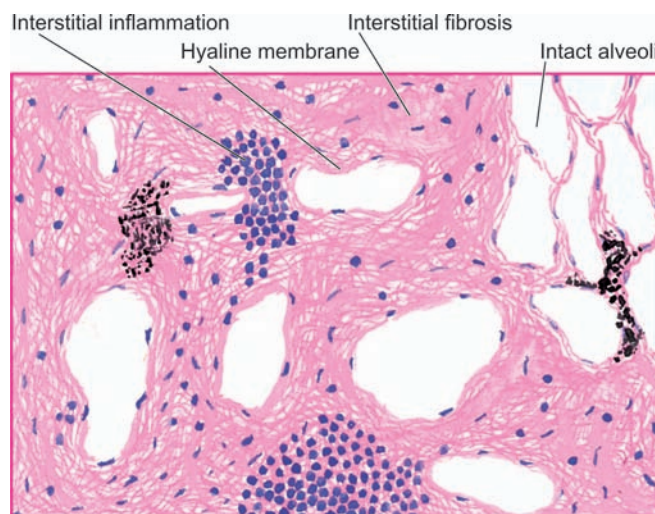


FIGURE 26.4

Microscopic appearance of interstitial pneumonitis (viral pneumonia). There are predominant inflammatory changes in the alveolar walls and pronounced interstitial fibrosis.

infection and its complications. Most cases of interstitial pneumonitis recover completely. In more severe cases, there may be interstitial fibrosis and permanent damage.

CLINICAL FEATURES. Majority of cases of interstitial pneumonitis initially have upper respiratory symptoms with fever, headache and muscle-aches. A few days later appears dry, hacking, non-productive cough with retrosternal burning due to tracheitis and bronchitis. Chest radiograph may show patchy or diffuse consolidation. Cold agglutinin titres in the serum are elevated in almost half the cases of mycoplasmal pneumonia, 20% cases of adenovirus infection but absent in other forms of viral pneumonia. Isolation of the etiologic agent, otherwise, is difficult.

LUNG CANCER

A number of benign and malignant tumours occur in the lungs but the primary lung cancer, commonly termed bronchogenic carcinoma, is the most common (95% of all primary lung tumours). The lung is also the commonest site for metastasis from carcinomas and sarcomas. A histologic classification of various benign and malignant tumours of lungs as recommended by the World Health Organisation is given in Table 26.3.

BRONCHOGENIC CARCINOMA

Though the term bronchogenic carcinoma is commonly used for cancer of the lungs, it includes carcinomas having bronchial as well as bronchiolar origin.

INCIDENCE. Bronchogenic carcinoma is the most common primary malignant tumour in men in industrialised nations and accounts for nearly one-third of all cancer deaths in both sexes. Currently, the incidence in females in the United States has already exceeded breast cancer as a cause of death in women. Cancer of the lung is a disease of middle and late life with peak incidence in 5th to 7th decades, after which there is gradual fall in its incidence.

ETIOPATHOGENESIS. The high incidence of lung cancer is associated with a number of etiologic factors, most important of which is *cigarette smoking*.

1. Smoking. The most important factor for rise in the incidence of bronchogenic carcinoma is tobacco smoking. About 80% of lung cancer occurs in active smokers. A number of evidences support the positive relationship of lung cancer with tobacco smoking:

i) Total dose: There is a direct statistical correlation between death rate from lung cancer and the total amount of cigarettes smoked e.g.

TABLE 26.3: Histological Classification of Lung Tumours.

I. EPITHELIAL TUMOURS

A. Benign

1. Papilloma
2. Adenoma

B. Dysplasia and carcinoma in situ

C. Malignant

Bronchogenic carcinoma

1. Squamous cell (epidermoid) carcinoma
2. Small cell carcinoma
 - i) Oat cell carcinoma
 - ii) Intermediate cell carcinoma
 - iii) Combined oat cell carcinoma
3. Adenocarcinoma
 - i) Acinar adenocarcinoma
 - ii) Papillary adenocarcinoma
 - iii) Bronchiolo-alveolar carcinoma
 - iv) Solid carcinoma with mucus formation
4. Large cell carcinoma
5. Adenosquamous carcinoma

Other carcinomas

1. Pulmonary neuroendocrine tumour (carcinoid tumour)
2. Bronchial gland carcinomas
 - i) Adenoid cystic carcinoma
 - ii) Mucoepidermoid carcinoma

II. SOFT TISSUE TUMOURS

(Fibroma, fibrosarcoma; leiomyoma, leiomyosarcoma; lipoma, chondroma, haemangioma, lymphangioma, granular cell myoblastoma)

III. PLEURAL TUMOURS

A. Benign mesothelioma

B. Malignant mesothelioma

IV. MISCELLANEOUS TUMOURS

1. Carcinosarcoma
2. Pulmonary blastoma
3. Malignant melanoma
4. Malignant lymphoma

V. SECONDARY TUMOURS

VI. TUMOUR-LIKE LESIONS

1. Hamartomas
2. Eosinophilic granuloma
3. Inflammatory pseudotumours

■ An average regular smoker has 10 times greater risk of developing lung cancer than a non-smoker.

■ The risk of smokers of more than 2 packs (40 cigarettes) per day for 20 years is 20 times greater.

■ Cessation of smoking by a regular smoker results in gradual decline in the chances of developing lung cancer. After 10 years of abstinence from smoking, the risk is not greater than in a non-smoker.

■ Pipe and cigar smokers, though have higher risk than non-smokers but are at lesser risk than cigarette smokers.

ii) Histologic alterations: The association of tobacco smoking is strongest for squamous cell carcinoma and small cell carcinoma of the lung. More than 90% of smokers have epithelial changes in the respiratory tract in the form of squamous metaplasia, dysplasia or carcinoma *in situ* (Fig. 26.5).

iii) Mechanism: How tobacco smoking causes lung cancer is not quite clear.

■ Analysis of the tar from cigarette smoke has revealed a number of known carcinogens (e.g. polycyclic aromatic hydrocarbons, nitrosamines) and tumour promoters (e.g. phenol derivatives).

■ In experimental animal studies, it has been possible to induce cancer by skin painting experiments with smoke-tar. However, it has not been possible to reproduce pattern of human respiratory tract cancer, probably because of the difficulty in reproducing human smoking methods in animals.

2. Atmospheric pollution. There is increased risk of developing bronchogenic carcinoma in nonsmokers living in industrialised and smoky cities than in the less polluted rural areas. It is possible that specific industrial pollutants may be at fault as evidenced by high rates for lung cancer in people living in the neighbourhood of petrochemical industries.

3. Occupational causes. There are number of well-established occupational causes of lung cancer. These include workers exposed to asbestos, radiation of all types, bis-ethers, nickel, beryllium, arsenic, metallic iron and iron-oxide. Some industrial carcinogens and

cigarette smoking have cocarcinogenic effect, particularly in uranium mines and asbestos workers.

4. Dietary factors. Susceptibility to respiratory cancers is increased in vitamin A deficiency. Smokers with low vitamin A intake have a greater risk of lung cancer than those with vitamin A-rich diet. The incidence of lung cancer is inversely related to socioeconomic level reflecting their dietary pattern.

5. Genetic factors. The risk of developing lung cancer in the relatives of lung cancer patients is two and half times greater than that in the general population. A few studies have suggested that ability to metabolise carcinogenic polycyclic aromatic hydrocarbons is under genetic control but the exact nature of genetic and familial influence is uncertain.

6. Chronic scarring. Peripheral adenocarcinomas occur more frequently in areas of chronic scarring caused by chronic inflammatory changes, old tuberculosis, asbestosis, chronic interstitial fibrosis, old infarcts and in scleroderma.

PATHOLOGIC CHANGES. Bronchogenic carcinoma can occur anywhere in the lung but the most common location is *hilar*, followed in descending frequency by *peripheral* type.

Grossly, these 2 main types show variation in appearance:

1. Hilar type (Fig. 26.6,A): Most commonly, the lung cancer arises in the main bronchus or one of its segmental branches in the hilar parts of the lung, more often on the right side. The tumour begins as a small roughened area on the bronchial mucosa at

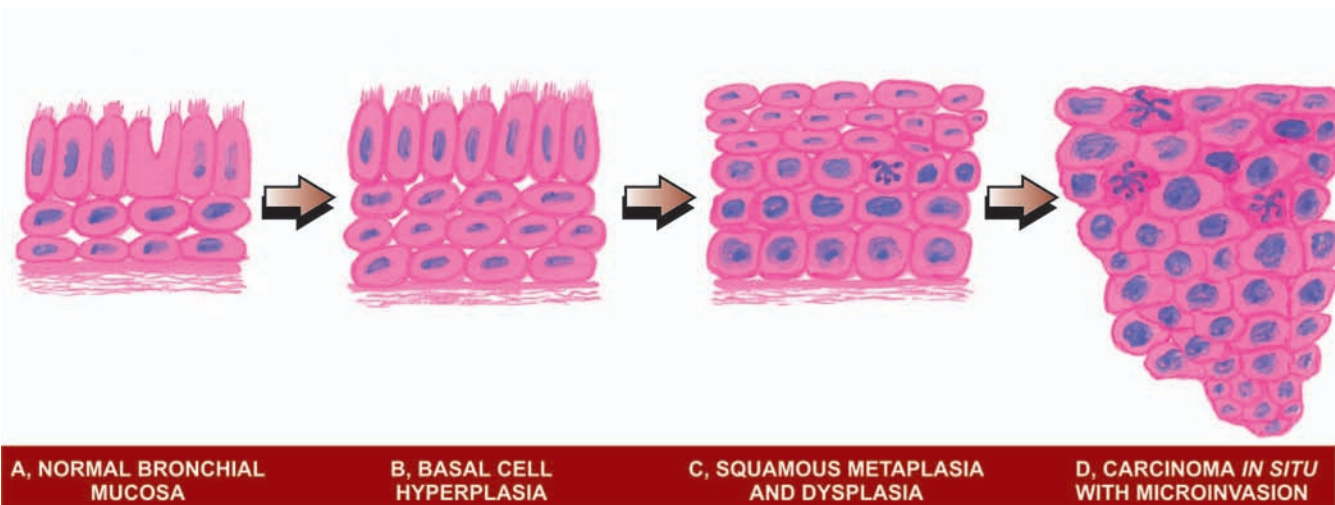
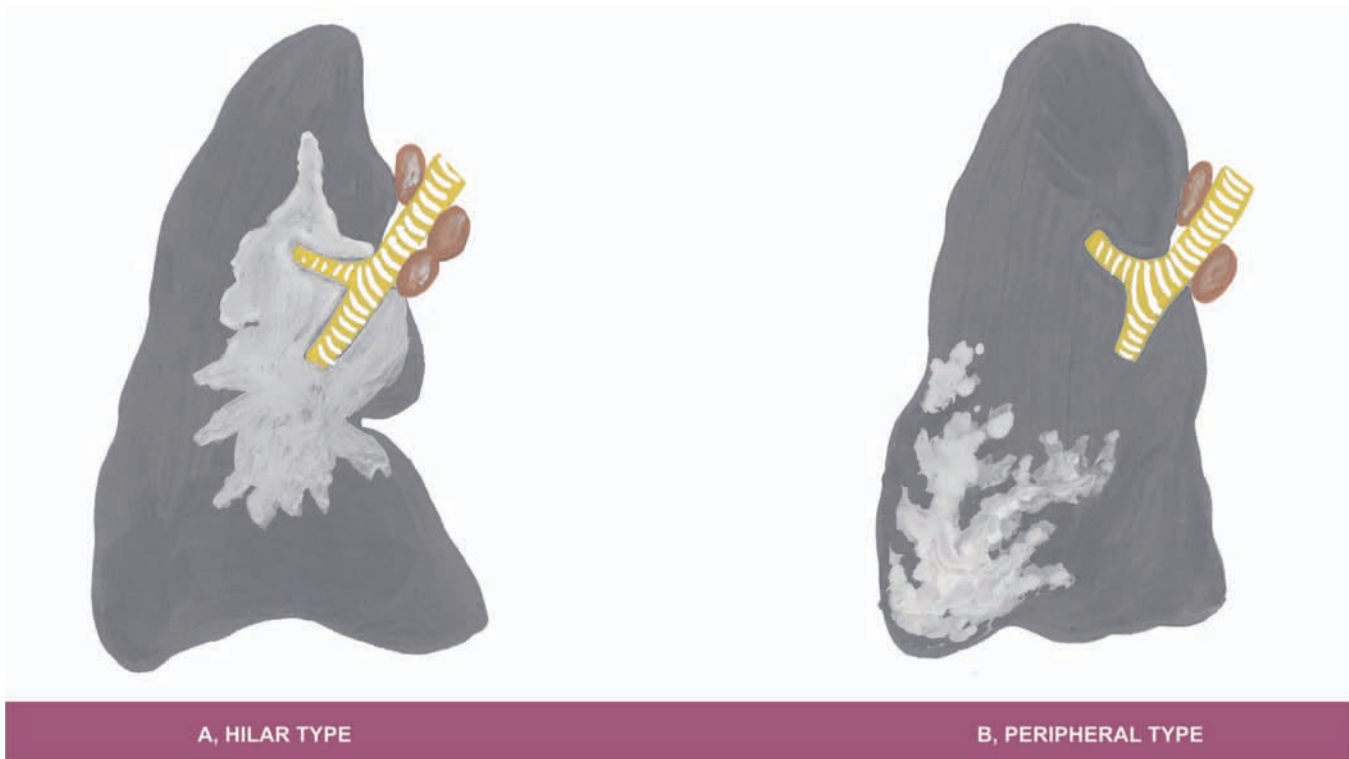


FIGURE 26.5

Schematic representation of sequential development of squamous cell carcinoma of the lung.

**FIGURE 26.6**

The two main gross patterns of bronchogenic carcinoma.

the bifurcation. As the tumour enlarges, it thickens the bronchial mucosa producing nodular or ulcerated surface. As the nodules coalesce, the carcinoma grows into a friable spherical mass, 1 to 5 cm in diameter, narrowing and occluding the lumen. The cut surface of the tumour is yellowish-white with foci of necrosis and haemorrhages which may produce cavitory lesions. It is common to find secondary changes in bronchogenic carcinoma of lung such as bronchopneumonia, abscess formation and bronchiectasis as a result of obstruction and intercurrent infections. The tumour soon spreads within the lungs by direct extension or by lymphatics, and to distant sites by lymphatic or haematogenous routes, as described later.

2. Peripheral type (Fig. 26.6,B): A small proportion of lung cancers, chiefly adenocarcinomas including bronchioloalveolar carcinomas, originate from a small peripheral bronchiole but the exact site of origin may not be discernible. The tumour may be a single nodule or multiple nodules in the periphery of the lung producing pneumonia-like consolidation of a large part of the lung. The cut surface of the tumour is greyish and mucoid.

Microscopically, as per the World Health Organisation recommendations (see Table 26.3), bronchogenic carcinoma is divided into 5 main histologic types: squamous cell or epidermoid carcinoma (35-50%), small cell carcinoma (20-25%), adenocarcinoma (15-35%), large cell carcinoma (10-15%), and combined squamous cell carcinoma and adenocarcinoma (adenosquamous carcinoma, 1-3%). However, for therapeutic purposes, bronchogenic carcinoma can be classified into three groups:

1. Small cell carcinoma (20-25%)
2. Non-small cell carcinomas (70-75%) (includes squamous cell carcinoma, adenocarcinoma, and large cell carcinoma)
3. Combined/mixed patterns (5-10%).

The distinction between small cell and non-small cell carcinomas is important because the two not only differ in morphology, but there are major differences in immunophenotyping and response to treatment:

■ **Immunophenotyping:** Small cell carcinomas generally have mutations in *TP53* and *RB* gene while non-small cell carcinomas have more often inactivation of *p16/CDK/N2A* genes.

■ **Response to treatment:** Small cell carcinomas frequently have haematogenous metastasis at the time of diagnosis and are treated by chemotherapy with or without radiation rather than by surgery, while non-small cell carcinomas respond poorly to chemotherapy and are better treated by surgery.

The major differences in small cell and non-small cell carcinomas of the lung are summed up in Table 26.4.

By light microscopy, precise histologic classification of bronchogenic carcinoma is possible which is important because of prognostic and therapeutic considerations.

1. Squamous cell (epidermoid) carcinoma (Fig. 26.7): This is the most common type of bronchogenic carcinoma found more commonly in men, particularly with history of tobacco smoking. These tumours usually arise in a large bronchus and are prone to massive necrosis and cavitation. The tumour is diagnosed microscopically by identification of either intercellular bridges or keratinisation. The tumour may show varying histologic grades of

differentiation such as well-differentiated, moderately-differentiated and poorly-differentiated (**COLOUR PLATE VII: CL 26**). Occasionally, a variant of squamous cell carcinoma, *spindle cell carcinoma*, having biphasic pattern of growth due to the presence of a component of squamous cell carcinoma and the other sarcoma-like spindle cell component, is found. Usually the spread of squamous cell carcinoma is more rapid than the other histologic types. Frequently, the edge of the growth and the adjoining uninvolved bronchi show squamous metaplasia, epithelial dysplasia and carcinoma *in situ*.

2. Small cell carcinoma: Small cell carcinomas are frequently hilar or central in location, have strong relationship to cigarette smoking and are highly malignant tumours. They are most often associated with ectopic hormone production because of the presence of neurosecretory granules in majority of tumour cells which are similar to those found in argentaffin or Kulchitsky cells normally found in bronchial epithelium. By immunohistochemistry, these tumour cells are positive for neuroendocrine

TABLE 26.4: Comparison of Features of Small Cell and Non-small Cell Carcinoma of the Lung.

FEATURE	SMALL CELL CARCINOMA	NON-SMALL CELL CARCINOMA
1. <i>Etiologic relationship</i>	Strongly related to tobacco smoking	Smoking implicated, other factors: pollution, chronic scars, asbestos exposure
2. <i>Morphology</i>		
i) <i>Pattern</i>	Diffuse sheets	Squamous or glandular pattern
ii) <i>Nuclei</i>	Hyperchromatic, fine chromatin	Pleomorphic, coarse chromatin
iii) <i>Nucleoli</i>	Indistinct	Prominent
iv) <i>Cytoplasm</i>	Scanty	Abundant
3. <i>Neuroendocrine markers</i> (e.g. dense-core granules on EM, chromogranin, synaptophysin, neuron-specific enolase, CD56, CD57)	Present	Absent
4. <i>Epithelial markers</i> (e.g. epithelial membrane antigen, carcinoembryonic antigen, cytokeratin)	Present	Present
5. <i>Mucin</i>	Absent	Present in adenocarcinoma
6. <i>HLA, β2 microglobulin</i>	Absent to low	Present
7. <i>Peptide hormone production</i>	Gastrin, ACTH, ADH, calcitonin	Parathormone
8. <i>Genetic abnormalities</i>	3p allele loss, RB and TP 53 mutations	3p allele loss, p16/CDKN2A and RAS mutations
9. <i>Treatment type</i>	Radiotherapy and/or chemotherapy	Surgical resection possible, limited response to radiotherapy and/or chemotherapy
10. <i>Prognosis</i>	Poor	Better

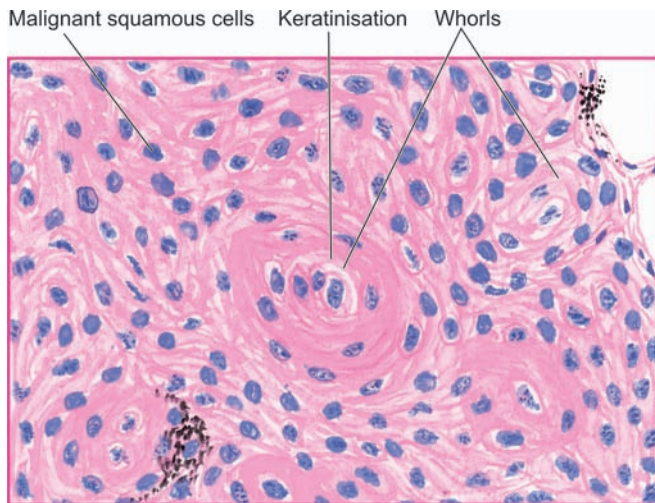


FIGURE 26.7

Squamous cell carcinoma of the lung. Islands of invading malignant squamous cells are seen. A few well-developed cell nests with keratinisation are evident.

markers: chromogranin, neuron-specific enolase (NSE) and synaptophysin. Small cell carcinomas have 3 subtypes:

i) *Oat cell carcinoma* (Fig. 26.8) is composed of uniform, small cells, larger than lymphocytes with dense, round or oval nuclei having diffuse chromatin, inconspicuous nucleoli and very sparse cytoplasm (*oat* = a form of grain). These cells are organised into cords, aggregates and ribbons or around small blood vessels forming pseudorosettes.

ii) *Small cell carcinoma, intermediate cell type* is composed of cells slightly larger than those of oat cell carcinoma and have similar nuclear characteristics but have more abundant cytoplasm. These cells are organised into lobules.

iii) *Combined oat cell carcinoma* is a tumour in which there is a definite component of oat cell carcinoma with squamous cell and/or adenocarcinoma.

3. Adenocarcinoma: Adenocarcinoma, also called *peripheral carcinoma* due to its location and *scar carcinoma* due to its association with areas of chronic scarring, is the most common bronchogenic carcinoma in women and is slow-growing. Adenocarcinoma is further subclassified into 4 types:

i) *Acinar adenocarcinoma* which has predominance of glandular structure and often occurs in the larger bronchi.

ii) *Papillary adenocarcinoma* which has a pronounced papillary configuration and is frequently peripherally

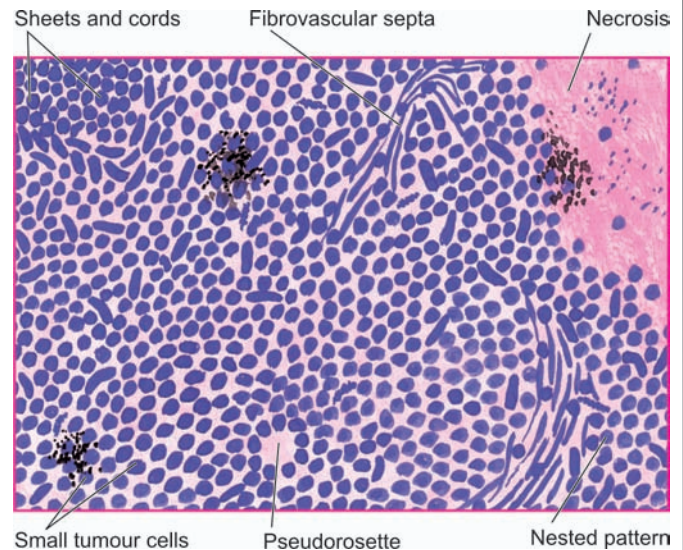


FIGURE 26.8

Oat cell carcinoma of the lung. The tumour cells are arranged in sheets, cords or aggregates and at places form pseudorosettes. The individual tumour cells are small, uniform, lymphocyte-like with scanty cytoplasm.

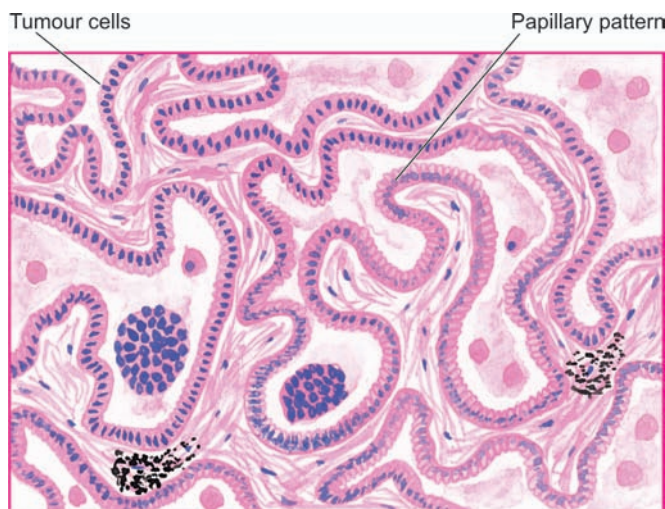
located in the lungs and is found in relation to pulmonary scars (scar carcinoma).

iii) *Bronchiolo-alveolar carcinoma* (Fig. 26.9) is characterised by cuboidal to tall columnar and mucus-secreting epithelial cells growing along the existing alveoli and forming numerous papillary structures. Ultrastructurally, these tumour cells resemble Clara cells or less often type II pneumocytes.

iv) *Solid carcinoma* is a poorly-differentiated adenocarcinoma lacking acini, tubules or papillae but having mucus-containing vacuoles in many tumour cells.

4. Large cell carcinoma: These are undifferentiated carcinomas which lack the specific features by which they could be assigned into squamous cell carcinoma or adenocarcinoma. Large cell carcinomas are more common in men, have strong association with cigarette smoking and are highly malignant tumours. The tumour cells have large nuclei, prominent nucleoli, abundant cytoplasm and well-defined cell borders. Variants of large cell undifferentiated carcinomas include *giant cell carcinoma* with prominence of highly pleomorphic multinucleate cells and *clear cell carcinoma* composed of cells with clear or foamy cytoplasm without mucin.

5. Adenosquamous carcinoma: These are a small proportion of peripheral scar carcinomas having clear

**FIGURE 26.9**

Bronchiolo-alveolar carcinoma. The alveolar walls are lined by cuboidal to tall columnar and mucin-secreting tumour cells with papillary growth pattern.

evidence of both keratinisation and glandular differentiation.

SPREAD. Bronchogenic carcinoma can invade the adjoining structures directly, or may spread by lymphatic and haematogenous routes.

1. Direct spread. The tumour extends directly by invading through the wall of the bronchus and destroys and replaces the peribronchial lung tissue. As it grows further, it spreads to the opposite bronchus and lung, into the pleural cavity, the pericardium and the myocardium and along the great vessels of the heart causing their constriction. Extension of the cancer located at the apex of the lung into the thoracic cage may involve brachial plexus and the sympathetic chain causing pain and sensory disturbances, so called *Pancoast's syndrome*.

2. Lymphatic spread. Initially, hilar lymph nodes are affected. Later, lymphatic metastases occur to the other groups leading to spread to mediastinal, cervical, supraclavicular and para-aortic lymph nodes. Invasion of the thoracic duct may produce chylous ascites.

3. Haematogenous spread. Distant metastases via blood stream are widespread and early. The sites affected, in descending order of involvement, are: the liver, adrenals, bones, pancreas, brain, opposite lung, kidneys and thyroid.

CLINICAL FEATURES. Symptoms of lung cancer are quite variable and result from local effects, effects due

to occlusion of a bronchus, local and distant metastases and paraneoplastic syndromes. The additional diagnostic aids include radiologic examination of the chest, cytologic examination of the sputum and bronchial washings.

1. Local symptoms. Most common local complaints are cough, chest pain, dyspnoea and haemoptysis.

A list of various causes of haemoptysis is given in Table 26.5.

2. Bronchial obstructive symptoms. Occlusion of a bronchus may result in bronchopneumonia, lung abscess and bronchiectasis in the lung tissue distal to the site of obstruction and cause their attendant symptoms like fever, productive cough, pleural effusion and weight loss.

3. Symptoms due to metastases. Distant spread may produce varying features and sometimes these are the first manifestation of lung cancer. These include: superior vena caval syndrome, painful bony lesions, paralysis of recurrent nerve and other neurologic manifestations resulting from brain metastases.

4. Paraneoplastic syndromes. A number of paraneoplastic syndromes (page 271) are associated with lung cancer. These include the following:

- i) Ectopic hormone production:** Different hormonal syndromes are characteristic of different histologic types of lung cancer. Small cell carcinomas are associated most often with ectopic hormone production. The various hormones elaborated by lung cancer are:
 - a) ACTH, producing Cushing's syndrome.

TABLE 26.5: Causes of Haemoptysis.**A. INFLAMMATORY**

1. Bronchitis
2. Bronchiectasis
3. Tuberculosis
4. Lung abscess
5. Pneumonias

B. NEOPLASTIC

1. Primary and metastatic lung cancer
2. Bronchial adenoma

C. OTHERS

1. Pulmonary thromboembolism
2. Left ventricular failure
3. Mitral stenosis
4. Trauma
5. Foreign bodies
6. Primary pulmonary hypertension
7. Haemorrhagic diathesis

- b) ADH, inducing hyponatraemia.
- c) Parathormone, causing hypercalcaemia.
- d) Calcitonin, producing hypocalcaemia.
- e) Gonadotropins, causing gynaecomastia.
- f) Serotonin, associated with carcinoid syndrome.

ii) Other systemic manifestations: These include the following:

- a) *Neuromuscular* e.g. polymyositis, myopathy, peripheral neuropathy and subacute cerebellar degeneration.
- b) *Skeletal* e.g. clubbing and hypertrophic osteoarthropathy.
- c) *Cutaneous* e.g. acanthosis nigricans and dermatomyopathy.
- d) *Cardiovascular* e.g. migratory thrombophlebitis (Trousseau's syndrome), nonbacterial thrombotic endocarditis.
- e) *Haematologic* e.g. abnormalities in coagulation and leukaemoid reaction.

STAGING AND PROGNOSIS. The widely accepted clinical staging of lung cancer is according to the TNM classification, combining features of primary Tumours, Nodal involvement and distant Metastases. TNM staging divides all lung cancers into the following 4 stages:

Occult: Malignant cells in the broncho-pulmonary secretions but no evidence of primary tumour or metastasis.

Stage I: Tumour less than 3 cm, with or without ipsilateral nodal involvement, no distant metastasis.

Stage II: Tumour larger than 3 cm, with ipsilateral hilar lymph node involvement, no distant metastasis.

Stage III: Tumour of any size, involving adjacent struc-

tures, involving contralateral lymph nodes or distant metastasis.

In general, tumour size larger than 5 cm has worse prognosis. Symptomatic patients, particularly with systemic symptoms, fare far badly than the non-symptomatic patients. The overall prognosis of bronchogenic carcinoma is dismal; 5-year survival rate with surgery combined with radiotherapy or chemotherapy is about 9%. Adenocarcinoma and squamous cell carcinoma which are localised, are resectable and have a slightly better prognosis. *Small cell carcinoma has the worst prognosis* since surgical treatment is ineffective though the tumour is sensitive to radiotherapy and chemotherapy.

METASTATIC LUNG TUMOURS

Secondary tumours of the lungs are more common than the primary pulmonary tumours. Metastases from carcinomas as well as sarcomas arising from anywhere in the body may spread to the lung by haematogenous or lymphatic routes, or by direct extension. Blood-borne metastases are the most common since emboli of tumour cells from any malignant tumour entering the systemic venous circulation are likely to be lodged in the lungs. Metastases are most common in the peripheral part of the lung forming single or multiple, discrete nodular lesions which appear radiologically as '*cannon-ball secondaries*'. Less frequently, the metastatic growth is confined to peribronchiolar and perivascular locations which is due to spread via lymphatics.

Most common sources of metastases in the lungs are: carcinomas of the bowel, breast, thyroid, kidney, pancreas, lung (ipsilateral or contralateral) and liver. Other tumours which frequently metastasise to the lungs are osteogenic sarcoma, neuroblastoma, Wilms' tumour, melanoma, lymphomas and leukaemias.



Diseases of Oral Cavity and Salivary Glands

ORAL SOFT TISSUES

NORMAL STRUCTURE

The oral cavity is the point of entry for digestive and respiratory tracts. The mucous membrane of the mouth consists of squamous epithelium covering vascularised connective tissue. The epithelium is keratinised over the hard palate, lips and gingiva, while elsewhere it is non-keratinised. Mucous glands (minor salivary glands) are scattered throughout the oral mucosa. Sebaceous glands are present in the region of the lips and the buccal mucosa only. Lymphoid tissue is present in the form of tonsils and adenoids.

The oral cavity is the site of numerous congenital and acquired diseases. Besides, many systemic diseases have oral manifestations. Some of the commonly occurring conditions are discussed here.

DEVELOPMENTAL ANOMALIES

1. FACIAL CLEFTS. *Cleft upper lip (harelip) and cleft palate*, alone or in combination, are the commonest developmental anomalies of the face. These occur from the failure of fusion of facial processes.

2. FORDYCE'S GRANULES. Fordyce's granules are symmetric, small, light yellow macular spots on the lips and buccal mucosa and represent collections of sebaceous glands. They remain undeveloped until puberty but occur quite commonly in adults.

3. LEUKOEDEMA. This is an asymptomatic condition occurring in children and is characterised by symmetric, grey-white areas on the buccal mucosa. *Microscopically*, there is pronounced intracellular oedema. There is no increased malignant potential compared to leukoplakia discussed below.

4. DEVELOPMENTAL DEFECTS OF THE TONGUE. These are as under:

i) **Macroglossia** is the enlargement of the tongue, usually due to lymphangioma or haemangioma, and sometimes due to amyloid tumour.

ii) **Microglossia and aglossia** are rare congenital anomalies representing small-sized and absence of tongue respectively.

iii) **Fissured tongue (scrotal, furrowed or grooved tongue)** is a genetically-determined condition characterised by numerous small furrows or grooves on the dorsum of the tongue. It is often associated with mild glossitis.

iv) **Bifid tongue** is a rare condition occurring due to failure of the two lateral halves of the tongue to fuse in the midline.

v) **Tongue tie** occurs when the lingual fraenum is quite short, or when the fraenum is attached near the tongue tip.

vi) **Hairy tongue** is not a true developmental defect, but is mentioned here alongwith other similar conditions. The filiform papillae are hypertrophied and elongated. These 'hairs' are stained black, brown or yellowish-white by food, tobacco, oxidising agents or by oral flora.

MUCOCUTANEOUS LESIONS

Lesions of the oral mucosa occur in many diseases of the skin. Some of these are as under:

1. LICHEN PLANUS. Characteristically, oral lichen planus appears as interlacing network of whitening or keratosis on the buccal mucosa.

2. VESICULAR LESIONS. A number of vesicular or bullous diseases of the skin have oral lesions.

i) **Pemphigus vulgaris.** Vesicular oral lesions appear invariably in all cases at some time in the course of pemphigus vulgaris. In about half the cases oral lesions are the initial manifestations.

ii) **Pemphigoid.** Vesicles or bullae appear on oral mucosa as well as on conjunctiva in pemphigoid and are seen more often in older women.

iii) **Erythema multiforme.** Subepithelial vesicles may occur on the skin as well as mucosae.

iv) **Stevens-Johnson syndrome** is a rather fatal and severe form of erythema multiforme involving oral and other mucous membranes occurring following ingestion of sulfa drugs.

v) **Epidermolysis bullosa** is a hereditary condition having subepidermal bullae on the skin as well as has oral lesions.

INFLAMMATORY DISEASES

1. **STOMATITIS.** Inflammation of the mucous membrane of the mouth is called stomatitis. It can occur in the course of many different diseases.

i) **Aphthous ulcers (Canker sores)** is the commonest form of oral ulceration. The etiology is unknown but may be precipitated by emotional factors, stress, allergy, hormonal imbalance, nutritional deficiencies, gastrointestinal disturbances, trauma etc. The condition is characterised by painful oral ulcers, 1 cm or more in size. Recurrent *aphthae* may form a part of Behcet's syndrome and inflammatory bowel disease.

ii) **Herpetic stomatitis** is an acute disease occurring in infants and young children. It is the most common manifestation of primary infection with herpes simplex virus. The lesions are in the form of vesicles around the lips. Similar lesions may appear on the genital skin. Recurrent attacks occur due to stress, emotional upsets and upper respiratory infections.

iii) **Necrotising stomatitis (Noma or Cancrum oris)** occurs more commonly in poorly-nourished children like in kwashiorkor; infectious diseases such as measles; immunodeficiencies and emotional stress. The lesions are characterised by necrosis of the marginal gingiva and may extend on to oral mucosa, causing cellulitis of the tissue of the cheek. The condition may progress to gangrene of the cheek.

iv) **Mycotic infections** commonly involving the oral mucosa are actinomycosis and candidiasis.

■ **Cervicofacial actinomycosis** is the commonest form of the disease developing at the angle of the mandible (Chapter 11).

■ **Candidiasis (moniliasis or thrush)** is caused by *Candida albicans* which is a commensal in the mouth. It appears as an opportunistic infection in immunocompromised host. There are erythematous lesions on the palate and angular cheilitis.

2. **GLOSSITIS.** **Acute glossitis** characterised by swollen papillae occurs in eruptions of measles and scarlet fever. In **chronic glossitis**, the tongue is raw and red without swollen papillae and is seen in malnutri-

tion such as in pellagra, ariboflavinosis and niacin deficiency. In iron deficiency anaemia, pernicious anaemia and sprue, there is *chronic atrophic glossitis* characterised by atrophied papillae and smooth raw tongue.

3. **SYPHILITIC LESIONS.** Oral lesions may occur in primary, secondary, tertiary and congenital syphilis (Chapter 11).

i) Extragenital chancre of *primary syphilis* occurs most commonly on the lips.

ii) *Secondary syphilis* shows maculopapular eruptions and mucous patches in the mouth.

iii) In the *tertiary syphilis*, gummas or diffuse fibrosis may be seen on the hard palate and tongue.

iv) Oral lesions of the *congenital syphilis* are fissures at the angles of mouth and characteristic peg-shaped notched Hutchinson's incisors (Chapter 11).

4. **TUBERCULOUS LESIONS.** Involvement of the mouth in tuberculosis is rare. The lesions are in the form of ulcers or elevated nodules.

5. **HIV INFECTION.** HIV infection of low grade as well as full-blown acquired immunodeficiency syndrome (AIDS) are associated with oral manifestations such as opportunistic infections, malignancy, hairy leukoplakia and others (Table 27.1). About half the cases of Kaposi's sarcoma have intraoral lesions as part of systemic involvement.

PIGMENTARY LESIONS

Oral and labial melanotic pigmentation may be observed in certain systemic and metabolic disorders such as

TABLE 27.1: Oral Manifestations of AIDS.

A. OPPORTUNISTIC INFECTIONS

<i>Fungal</i>	:	Candidiasis (oral thrush) Histoplasmosis Cryptococcosis
<i>Bacterial</i>	:	Dental caries and periodontitis Mycobacterial infections
<i>Viral</i>	:	Herpetic stomatitis Cytomegalovirus Human papilloma virus

B. TUMOURS

Kaposi's sarcoma
Squamous cell carcinoma
Non-Hodgkin's lymphoma

C. OTHERS

Hairy leukoplakia
Recurrent aphthous ulcers

Addison's disease, Albright syndrome, Peutz-Jeghers syndrome and haemochromatosis. All types of pigmented naevi as well as malignant melanoma can occur in oral cavity. Exogenous pigmentation such as due to deposition of lead sulfide can also occur.

TUMOURS AND TUMOUR-LIKE LESIONS

Benign and malignant tumours of various types and also a number of tumour-like lesions and premalignant lesions are encountered in the oral soft tissues. A list of such lesions is presented in Table 27.2.

A. TUMOUR-LIKE LESIONS

A number of proliferative lesions arising from the oral tissues are tumour-like masses which clinically may resemble neoplasms. Some of these are as under:

1. FIBROUS GROWTHS. Fibrous growths of the oral soft tissues are very common. These are not true tumours (unlike intraoral fibroma and papilloma), but are instead inflammatory or irritative in origin. A few common varieties are as under:

i) Fibroepithelial polyps occur due to irritation or chronic trauma. These are composed of reparative fibrous tissue, covered by a thin layer of stratified squamous epithelium.

ii) Fibrous epulis is a lesion occurring on the gingiva and is localised hyperplasia of the connective tissue following trauma or inflammation in the area.

iii) Denture hyperplasia occurs in edentulous or partly edentulous patients. The lesion is an inflammatory hyperplasia in response to local irritation by ill-fitting denture or an elongated tooth.

2. PYOGENIC GRANULOMA. This is an elevated, bright red swelling of variable size occurring on the lips, tongue, buccal mucosa and gingiva. It is a vaso-proliferative inflammatory lesion. *Pregnancy tumour* is a variant of pyogenic granuloma.

3. MUCOCELE. Also called mucous cyst, it is a cystic dilatation of the mucous glands of the oral mucosa. The cyst often ruptures on distension and incites inflammatory reaction.

4. RANULA. It is a large mucocele located on the floor of the mouth. The cyst is lined by true epithelial lining.

5. DERMOID CYST. This tumour-like mass in the floor of the mouth represents a developmental malformation. The cyst is lined by stratified squamous epithelium. The cyst wall contains sebaceous glands, sweat glands, hair follicles and other mature tissues.

TABLE 27.2: Classification of Tumours and Tumour-like Lesions of the Oral Soft Tissues.

A. TUMOUR-LIKE LESIONS

1. Fibrous growths
(Fibroepithelial polyps, fibrous epulis, denture hyperplasia)
2. Pyogenic granuloma
3. Mucocele
4. Ranula
5. Dermoid cyst

B. BENIGN TUMOURS

1. Squamous papilloma
2. Haemangioma
3. Lymphangioma
4. Fibroma
5. Fibromatosis gingivae
6. Tumours of minor salivary glands
(e.g. Pleomorphic adenoma)
7. Granular cell myoblastoma
8. Other rare benign tumours

C. PREMALIGNANT LESIONS

1. Hyperkeratotic leukoplakia
2. Dysplastic leukoplakia

D. MALIGNANT TUMOURS

1. Squamous cell (Epidermoid) carcinoma
2. Other malignant tumours

B. BENIGN TUMOURS

Different parts of the mouth have a variety of mesodermal tissues and keratinising and non-keratinising epithelium. Therefore, the majority of neoplasms arising from the oral tissues are just like their counterparts in other parts of the body. Some of the common benign tumours of the mouth are as under:

1. SQUAMOUS PAPILLOMA. Papilloma can occur anywhere in the mouth and has the usual papillary or finger-like projections.

Microscopically, each papilla is composed of vascularised connective tissue covered by squamous epithelium.

2. HAEMANGIOMA. Haemangioma can occur anywhere in the mouth; when it occurs on tongue it may cause macroglossia. It is most commonly capillary type, although cavernous and mixed types may also occur (**COLOUR PLATE IX: CL 34**).

3. LYMPHANGIOMA. Lymphangioma may develop most commonly on the tongue producing macroglossia; on the lips producing macrocheilia, and on the cheek.

Cystic hygroma is a special variety of lymphangioma occurring in children on the lateral side of neck.

Microscopically, lymphangioma is characterised by large lymphatic spaces lined by endothelium and containing lymph (page 295).

4. FIBROMA. The true fibroma of mouth is relatively uncommon benign tumour and is more often a tumour-like lesion (discussed above).

Microscopically, fibroma is composed of collagenic fibrous connective tissue covered by stratified squamous epithelium.

5. FIBROMATOSIS GINGIVAE. This is a fibrous overgrowth of unknown etiology involving the entire gingiva. Sometimes the fibrous overgrowth is so much that the teeth are covered by fibrous tissue.

6. TUMOURS OF MINOR SALIVARY GLANDS. Minor salivary glands present in the oral cavity may sometimes be the site of origin of salivary tumours similar to those seen in the major salivary glands (page 352). Pleomorphic adenoma is a common example.

7. GRANULAR CELL MYOBLASTOMA. This is an unusual oral benign tumour, seen more often in the tongue.

Microscopically, the tumour is composed of large polyhedral cells with granular, acidophilic cytoplasm. The covering epithelium usually shows pronounced pseudoepitheliomatous hyperplasia.

8. OTHER RARE BENIGN TUMOURS. Some other rare benign tumours which can occur in the oral soft tissues are: neurilemmoma, neurofibroma, lipoma, giant cell granuloma, rhabdomyoma, leiomyoma, solitary plasmacytoma, osteoma, chondroma, naevi and vascular oral lesions seen in hereditary haemorrhagic telangiectasia (Osler-Rendu-Weber syndrome) and encephalofacial angiomatosis (Sturge-Weber syndrome).

C. ORAL LEUKOPLAKIA (WHITE LESIONS)

DEFINITION. Leukoplakia (*white plaque*) may be clinically defined as a white patch or plaque on the oral mucosa, exceeding 5 mm in diameter, which cannot be rubbed off nor can be classified into any other diagnosable disease. A number of other lesions are characterised by the formation of white patches listed in Table 27.3. However, from the pathologist's point of view, the term 'leukoplakia' is reserved for epithelial thickening

TABLE 27.3: Causes of White Lesions in the Oral Mucosa.

A. BENIGN

1. Fordyce's granules
2. Hairy tongue
3. Leukoedema
4. Lupus erythematosus
5. White sponge naevus

B. PREMALIGNANT

1. Leukoplakia
2. Oral lichen planus

C. MALIGNANT

Squamous cell carcinoma

which may range from completely benign to atypical and to premalignant cellular changes.

INCIDENCE. It occurs more frequently in males than females. The lesions may be of variable size and appearance. The sites of predilection, in descending order of frequency, are: cheek mucosa, angles of mouth, alveolar mucosa, tongue, lip, hard and soft palate, and floor of the mouth. In about 4-6% cases of leukoplakia, carcinomatous change is reported. However, it is difficult to decide which white lesions may undergo malignant transformation, but speckled or nodular form is more likely to progress to malignancy. Therefore, it is desirable that all oral white patches be biopsied to exclude malignancy.

ETIOLOGY. The etiological factors are similar to those suggested for carcinoma of the oral mucosa. *It has the strongest association with the use of tobacco in various forms*, e.g. in heavy smokers (especially in pipe and cigar smokers) and improves when smoking is discontinued, and in those who chew tobacco as in paan, paan masaala, zarda, gutka etc. The condition is also known by other names such as *smokers keratosis* and *stomatitis nicotina*. Other etiological factors implicated are chronic friction such as with ill-fitting dentures or jagged teeth, and local irritants like excessive consumption of alcohol and very hot and spicy foods and beverages. A special variety of leukoplakia called '*hairy leukoplakia*' has been described in patients of AIDS and has hairy or corrugated surface but is not related to development of oral cancer.

PATHOLOGIC CHANGES. *Grossly*, the lesions of leukoplakia may appear white, whitish-yellow, or red-velvety of more than 5 mm diameter and variable in appearance. They are usually circumscribed, slightly elevated, smooth or wrinkled, speckled or nodular.

Microscopically, leukoplakia is of 2 types:

1. Hyperkeratotic type. This is characterised by an orderly and regular hyperplasia of squamous epithelium with hyperkeratosis on the surface (**COLOUR PLATE IX: CL 33**).

2. Dysplastic type. When the changes such as irregular stratification of the epithelium, focal areas of increased and abnormal mitotic figures, hyperchromatism, pleomorphism, loss of polarity and individual cell keratinisation are present, the lesion is considered as epithelial dysplasia. The subepithelial tissues usually show an inflammatory infiltrate composed of lymphocytes and plasma cells. The extent and degree of the epithelial changes indicate the degree of severity of the epithelial dysplasia. Usually, mild dysplasia may revert back to normal if the offending etiologic factor is removed, whereas severe dysplasia indicates that the case may progress to carcinoma. *Erythroplasia* is a form of dysplastic leukoplakia in which the epithelial atypia is more marked and thus has higher risk of developing malignancy. If the epithelial dysplasia is extensive so as to involve the entire thickness of the epithelium, the lesion is called carcinoma *in situ* which may progress to invasive carcinoma (Fig. 27.1).

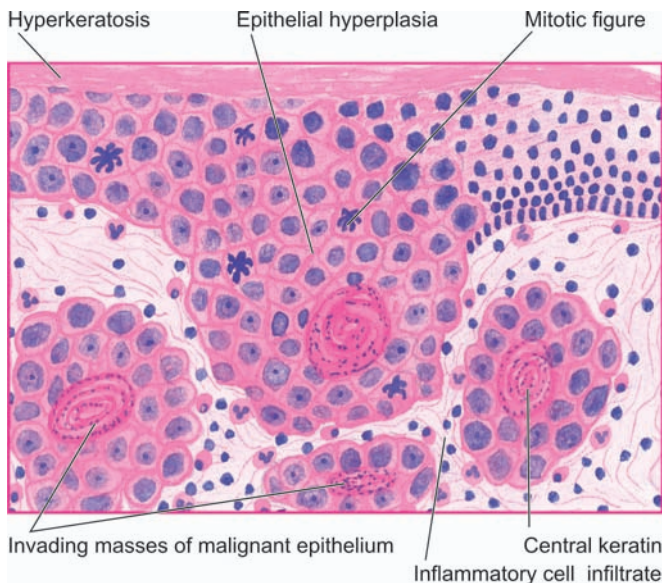


FIGURE 27.1

Oral mucosa showing epithelial dysplasia progressing to invasive squamous cell carcinoma. There is keratosis, irregular stratification, cellular pleomorphism, increased and abnormal mitotic figures and individual cell keratinisation, while a few areas show superficial invasive islands of malignant cells in the subepithelial soft tissues.

D. MALIGNANT TUMOURS

Squamous Cell (Epidermoid) Carcinoma

Oral cancer is a disease with very poor prognosis because it is not recognised and treated when small and early.

INCIDENCE. Squamous cell (epidermoid) carcinoma comprises 90% of all oral malignant tumours and 5% of all human malignancies. The peak incidence in the UK and the USA is from 55 to 75 years of age, whereas in India it is from 40 to 45 years of age. Oral cancer is a very frequent malignancy in India, Sri Lanka and some Eastern countries, probably related to habits of betel-nut chewing and reversed smoking (page 248). There is a definite male preponderance. It can occur anywhere in the mouth but certain sites are more commonly involved. These sites, in descending order of frequency, are: the lips (more commonly lower), tongue, anterior floor of mouth, buccal mucosa in the region of alveolar lingual sulcus, and palate (Fig. 27.2).

ETIOLOGY. As with other forms of cancer, the etiology of squamous cell carcinoma is unknown. But a number of etiological factors have been implicated:

Strong association:

- Tobacco smoking and tobacco chewing causing leukoplakia is the most important factor as discussed above.
- Chronic alcohol consumption.
- Human papilloma virus infection, particularly HPV 16, 18 and 33 types.

Weak association:

- Chronic irritation from ill-fitting denture or jagged teeth.

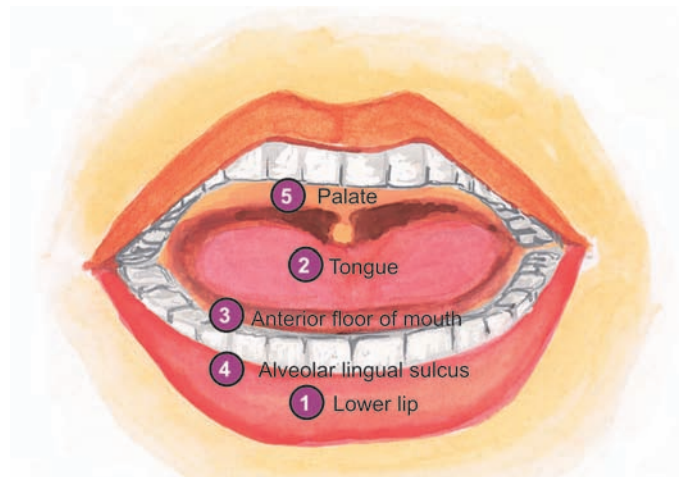


FIGURE 27.2

Frequency of occurrence of squamous cell carcinomas in the oral cavity.

- ii) Submucosal fibrosis as seen in Indians consuming excess of chillies.
- iii) Poor orodental hygiene.
- iv) Nutritional deficiencies.
- v) Exposure to sunlight (in relation to lip cancer).
- vi) Exposure to radiation.
- vii) Plummer-Vinson syndrome, characterised by atrophy of the upper alimentary tract.

PATHOLOGIC CHANGES. *Grossly*, squamous cell carcinoma of oral cavity may have the following types (Fig. 27.3):

i) Ulcerative type—is the most frequent type and is characterised by indurated ulcer and firm everted or rolled edges.

ii) Papillary or verrucous type—is soft and wartlike growth.

iii) Nodular type—appears as a firm, slow growing submucosal nodule.

iv) Scirrhus type—is characterised by infiltration into deeper structures.

All these types may appear on a background of leukoplakia or erythroplasia of the oral mucosa. Enlarged cervical lymph nodes may sometimes be present.

Microscopically, squamous cell carcinoma ranges from well-differentiated keratinising carcinoma to highly-undifferentiated neoplasm. Changes of epithelial dysplasia are often present in the surrounding areas of the lesion. Carcinoma of the lip and intraoral squamous carcinoma are usually always well-differentiated (see Fig. 27.1) (COLOUR PLATE VII: CL 26).

Carcinoma of the lip has a more favourable prognosis due to visible and easily accessible location and less frequent metastasis to the regional lymph

nodes. However, intraoral squamous carcinomas have poor prognosis because they are detected late and metastasis to regional lymph nodes occur early, especially in the case of carcinoma of tongue and soft palate. *Verrucous carcinoma*, on the other hand, is composed of very well-differentiated squamous epithelium with minimal atypia and hence has very good prognosis.

OTHER MALIGNANT TUMOURS

Other less common malignant neoplasms which may be encountered in the oral cavity are: malignant melanoma, lymphoepithelial carcinoma, malignant lymphoma, malignant tumours of minor salivary glands, and various sarcomas like rhabdomyosarcoma, liposarcoma, alveolar soft part sarcoma, Kaposi's sarcoma and fibrosarcoma. Metastatic tumours can also occur in the soft tissues of the mouth.

TEETH AND PERIODONTAL TISSUES

NORMAL STRUCTURE

The teeth are normally composed of 3 calcified tissues, namely: enamel, dentine and cementum; and the pulp composed of connective tissue. The teeth are surrounded by the portion of oral mucosa called the gingiva or gum (Fig. 27.4).

Enamel is the outer covering of teeth composed almost entirely of inorganic material (as in bone) which can be demonstrated in ground sections only as it is lost in decalcified section.

Dentine lies under the enamel and comprises most of the tooth substance. It is composed of organic material in the form of collagen fibrils as well as inorganic material in the form of calcium phosphates as in bone. Dentine is composed of odontoblasts or dentine cells which are counterparts of osteocytes in bone but differ from the latter in having odontoblast processes.

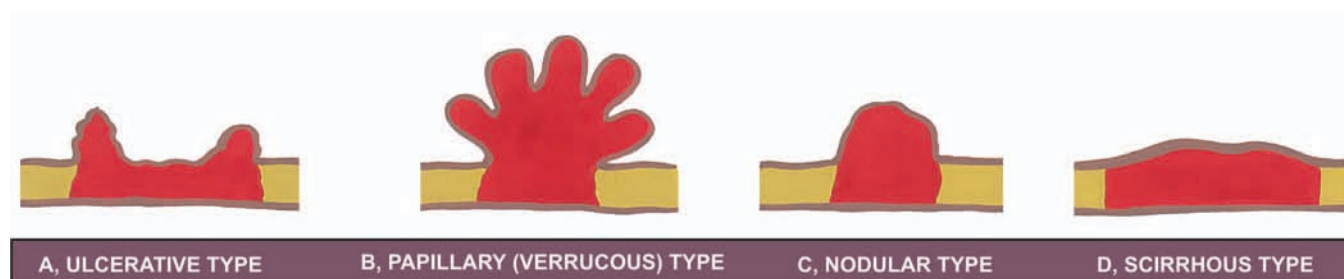


FIGURE 27.3

Squamous cell (Epidermoid) carcinoma of oral cavity, patterns of gross appearance.

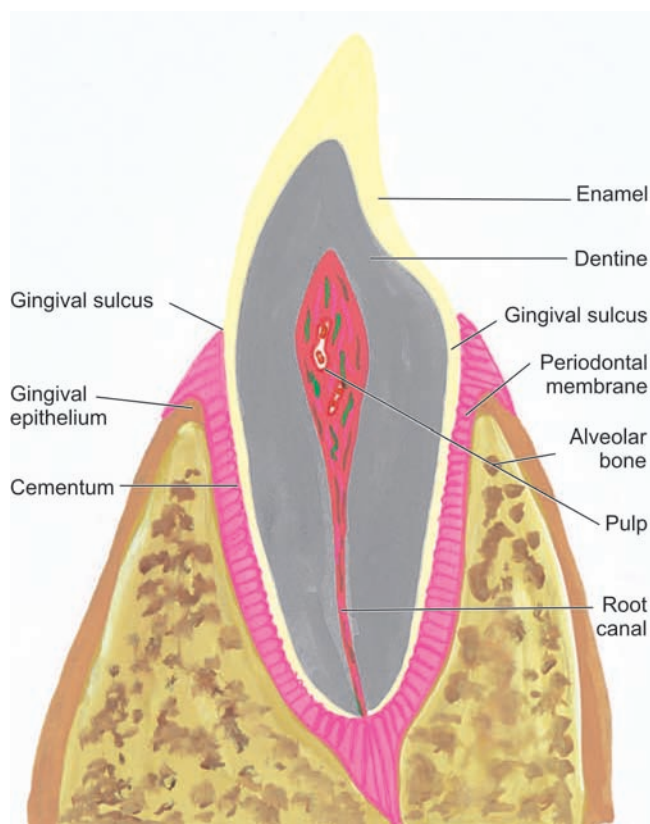


FIGURE 27.4

The normal structure of tooth embedded in the jaw.

Cementum is the portion of tooth which covers the dentine at the root of tooth and is the site where periodontal ligament is attached. Cementum is similar to bone in morphology and composition.

Dental pulp is inner to dentine and occupies the pulp cavity and root canal. It consists of connective tissue, blood vessels and nerves.

DENTAL CARIES

Dental caries is the most common disease of dental tissues, causing destruction of the calcified tissues of the teeth.

ETIOPATHOGENESIS. Dental caries is essentially a disease of modern society, associated with diet containing high proportion of refined carbohydrates. It has been known for almost 100 years that mixture of sugar or bread with saliva in the presence of acidogenic bacteria of the mouth, especially streptococci, produces organic acids which can decalcify enamel and dentine. Enamel is largely composed of inorganic material which virtually disintegrates. Dentine contains organic material

also which is left after decalcification. Bacteria present in the oral cavity cause proteolysis of the remaining organic material of dentine so that the process of destruction is complete. Diets rich in carbohydrates do not require much chewing and thus the soft and sticky food gets clung to the teeth rather than being cleared away, particularly in the areas of occlusal pits and fissures. 'Bacterial plaques' are formed in such stagnation areas. If these plaques are not removed by brushing or by vigorous chewing of fibrous foods, the process of tooth decay begins. There is evidence that consumption of water containing one part per million (ppm) fluoride is sufficient to reduce the rate of tooth decay in children.

PATHOLOGIC CHANGES. Caries occurs chiefly in the areas of pits and fissures, mainly of the molars and premolars, where food retention occurs, and in the cervical part of the tooth.

Macroscopically, the earliest change is the appearance of a small, chalky-white spot on the enamel which subsequently enlarges and often becomes yellow or brown and breaks down to form carious cavity. Eventually, the cavity becomes larger due to fractures of enamel. Once the lesion reaches enamel-dentine junction, destruction of dentine also begins. **Microscopically**, inflammation (pulpitis) and necrosis of pulp take place. There is evidence of reaction of the tooth to the carious process in the form of *secondary dentine*, which is a layer of odontoblasts laid down under the original dentine (Fig. 27.5).

SEQUELAE OF CARIES. Carious destruction of dental hard tissues frequently produces pulpitis and other inflammatory lesions like apical granuloma and apical abscess. Other less common causes of these lesions are



FIGURE 27.5

Dental caries. There is complete destruction of enamel, deposition of secondary dentine and evidence of pulpitis.

fracture of tooth and accidental exposure of pulp by the dentist.

1. Pulpitis. Pulpitis may be acute or chronic.

■ *Acute pulpitis* is accompanied by severe pain which may be continuous, throbbing or dull, and is accentuated by heat or cold. It is often accompanied by mild fever and leucocytosis.

■ *Chronic pulpitis* occurs when pulp is exposed widely. It is often not associated with pain. Chronically inflamed pulp tissue may protrude through the cavity forming polyp of the pulp. It may be partly covered by implanted squamous epithelium.

2. Apical granuloma. Pulpitis may lead to spread of infection through the apical foramen into the tissues surrounding the root of the tooth.

Microscopically, there is chronic inflammatory reaction with formation of granulation tissue and inclusion of nests or strands of squamous epithelium derived from remnants of odontogenic epithelium normally present in the periodontal membrane. An apical granuloma may develop into a dental (radicular) cyst as discussed below.

3. Apical abscess. An apical granuloma or acute pulpitis may develop into apical abscess. Acute abscess is very painful, while pus in chronic abscess may escape through root canal and cause further complications like osteomyelitis, cellulitis, cerebral abscess, meningitis and cavernous sinus thrombosis.

PERIODONTAL DISEASE

Chronic inflammation and degeneration of the supporting tissues of teeth resulting in teeth loss is a common condition. Besides inflammation, two other diseases—leukaemia and scurvy, are associated with gingival swelling.

The inflammatory periodontal disease affects adults more commonly. Pregnancy, puberty and use of drugs like dilantin are also associated with periodontal disease more often. The disease begins as *chronic marginal gingivitis*, secondary to bacterial plaques around the teeth such as due to calculus (tartar) on the tooth surface, impacted food, uncontrolled diabetes, tooth-decay and ill-fitting dental appliances. The gingival sulcus acts as convenient site for lodgement of food debris and bacterial plaque leading to formation of periodontal pocket from which purulent discharge can be expressed by digital pressure.

Pathologically, chronic marginal gingivitis is characterised by heavy chronic inflammatory cell infiltrate,

destruction of collagen, and epithelial hyperplasia so as to line the pocket. Untreated chronic marginal gingivitis slowly progresses to *chronic periodontitis* or *pyorrhoea* in which there is inflammatory destruction of deeper tissues. At this stage, progressive resorption of alveolar bone occurs and the tooth ultimately gets detached.

EPITHELIAL CYSTS OF JAW

The epithelium-lined cysts of dental tissue can have inflammatory or developmental origin. A classification of such cysts is given in Table 27.4.

A. INFLAMMATORY CYSTS

Radicular Cyst

Radicular cyst, also called as apical, periodontal or simply dental cyst, is the most common cyst originating from the dental tissues. It arises consequent to inflammation following destruction of dental pulp such as in dental caries, pulpitis, and apical granuloma. The epithelial cells of Mallasez, which normally lie in the periodontal ligament, proliferate within apical granuloma under the influence of inflammation, leading to the formation of an epithelium-lined cystic cavity. Most often, radicular cyst is observed at the apex of an erupted tooth and sometimes contains thick pultaceous material.

Microscopically, the radicular cyst is lined by nonkeratinised squamous epithelium. Epithelial rete processes may penetrate the underlying connective tissues. Radicular cyst of maxilla may be lined by respiratory epithelium. The cyst wall is fibrous and contains chronic inflammatory cells (lymphocytes,

TABLE 27.4: Classification of Epithelial Cysts of Jaw.

A. INFLAMMATORY

Radicular (apical, periodontal, dental) cyst

B. DEVELOPMENTAL

1. Odontogenic cysts

- (i) Dentigerous (follicular) cyst
- (ii) Eruption cyst
- (iii) Gingival cyst
- (iv) Primordial cyst (odontogenic keratocyst)

2. Non-odontogenic and fissural cysts

- (i) Nasopalatine duct (Incisive canal, Median anterior maxillary) cyst
- (ii) Nasolabial (nasoalveolar) cyst
- (iii) Globulomaxillary cyst
- (iv) Dermoid cyst

plasma cells with Russell bodies and macrophages) hyaline bodies and deposits of cholesterol crystals which may be associated with foreign body giant cells (Fig. 27.6).

B. DEVELOPMENTAL CYSTS

1. Odontogenic Cysts

I) DENTIGEROUS (FOLLICULAR) CYST. Dentigerous cyst arises from enamel of an unerupted tooth. The mandibular third molars and the maxillary canines are most often involved. Dentigerous cysts are less common than radicular cysts and occur more commonly in children and young individuals. These cysts are more significant because of reported occurrence of ameloblastoma and carcinoma in them.

Microscopically, dentigerous cyst is composed of a thin fibrous tissue wall lined by stratified squamous epithelium. Thus, the cyst may resemble radicular cyst, except that chronic inflammatory changes so characteristic of radicular cyst, are usually absent in dentigerous cyst (Fig. 27.7).

II) ERUPTION CYST. This is a cyst lying over the crown of an unerupted tooth and is lined by stratified squamous epithelium. It is thus a form of dentigerous cyst.

III) GINGIVAL CYST. It arises from the epithelial rests in the gingiva and is lined by keratinising squamous epithelium.

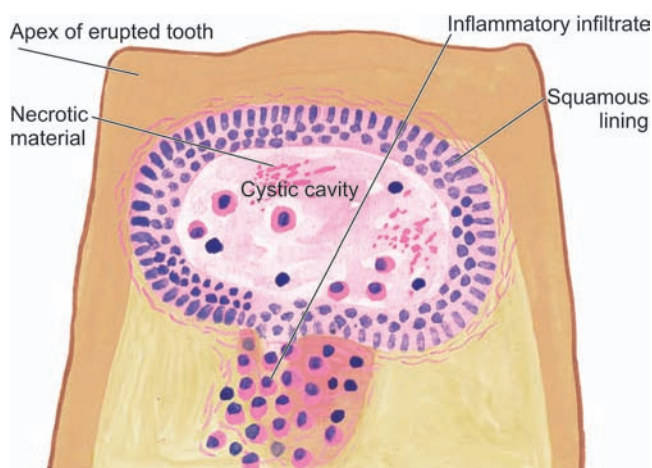


FIGURE 27.6

Dental (radicular) cyst. The cyst wall is composed of fibrous tissue and is lined by non-keratinised squamous epithelium. The cyst wall is densely infiltrated by chronic inflammatory cells, chiefly lymphocytes, plasma cells and macrophages.

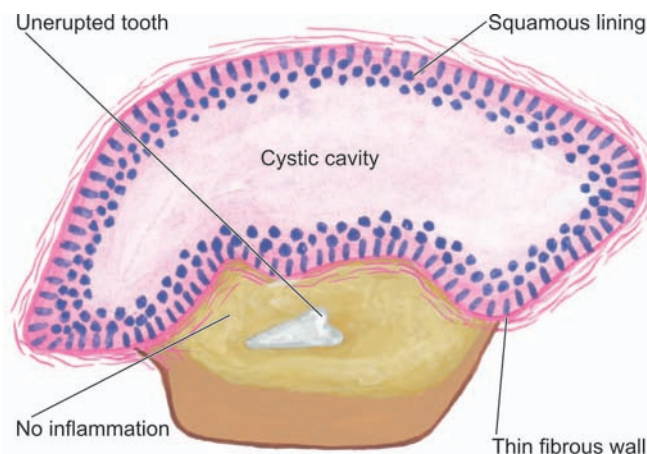


FIGURE 27.7

Dentigerous (Follicular) cyst. The cyst is composed of thin fibrous tissue wall and is lined by stratified squamous epithelium. A partly formed unerupted tooth is also seen in the wall. Inflammatory changes are conspicuously absent.

IV) PRIMORDIAL CYST (ODONTOGENIC KERATOCYST). Primordial cyst, like dentigerous cyst, also arises from tooth-forming epithelium. The common location is mandibular third molar.

Microscopically, the cyst wall is thin and is lined by regular layer of keratinising stratified squamous epithelium. Inflammatory changes are normally absent. Primordial cysts have a marked tendency to recur (50%). Multiple primordial cysts occur in association with naevoid basal cell carcinoma syndrome.

2. Non-odontogenic and Fissural Cysts

I) NASOPALATINE DUCT (INCISIVE CANAL, MEDIAN, ANTERIOR MAXILLARY) CYST. This is the most common non-odontogenic (fissural) cyst and arises from the epithelial remnants of the nasopalatine duct.

Microscopically, the cyst is lined by stratified squamous epithelium, respiratory epithelium, or both.

II) NASOLABIAL (NASOALVEOLAR) CYST. This cyst is situated in the soft tissues at the junction of median nasal, lateral nasal and maxillary processes, at the ala of the nose, and sometimes extending into the nostril.

Microscopically, the cyst is lined by squamous or respiratory epithelium, or both.

III) GLOBULOMAXILLARY CYST. This is an intra-osseous cyst and is rare.

IV) DERMOID CYST. The dermoid cyst is common in the region of head or neck, especially in the floor of the mouth. The cyst arises from remains in the midline during closure of mandibular and branchial arches.

ODONTOGENIC TUMOURS

Odontogenic tumours are a group of uncommon lesions of jaw derived from the odontogenic apparatus. These tumours are usually benign but some have malignant counterparts. A brief classification modified from the WHO classification is presented in Table 27.5.

A. BENIGN ODONTOGENIC TUMOURS

Ameloblastoma

Ameloblastoma is the most common benign but locally invasive epithelial odontogenic tumour. It is commonest in the 3rd to 5th decades of life. Preferential sites are the mandible in the molar-ramus area and the maxilla. The tumour originates from dental epithelium of the enamel itself or its epithelial residues. Sometimes, the tumour may arise from the epithelial lining of a dentigerous cyst or from basal layer of oral mucosa. Radiologically, typical picture is of a multilocular destruction of bone. Rare instances of an extraosseous example, presence of an embedded tooth, or unilocular ameloblastoma can occur. Tumour with histologic resemblance

to ameloblastoma can occur occasionally in the long bone, like adamantinoma of the tibia (page 425).

Grossly, the tumour is greyish-white, usually solid, sometimes cystic, replacing and expanding the affected bone.

Histologically, ameloblastoma can show different patterns described below (COLOUR PLATE IX: CL 35):

i) *Follicular pattern* is the most common. The tumour consists of follicles of variable size and shape and separated from each other by fibrous tissue. The structure of follicles is similar to that of enamel organ consisting of central area of stellate cells resembling stellate reticulum, and peripheral layer of cuboidal or columnar cells resembling epithelium. The central stellate areas may show cystic changes.

ii) *Plexiform pattern* is the next common pattern after follicular pattern. The tumour epithelium is seen to form irregular plexiform masses or network of strands. The stroma is usually scanty. Microcyst formation can occur in the stroma.

iii) *Acanthomatous pattern* is squamous metaplasia within the islands of tumour cells.

iv) *Basal cell pattern* of ameloblastoma is similar to basal cell carcinoma of the skin.

v) *Granular cell pattern* is characterised by appearance of acidophilic granularity in the cytoplasm of tumour cells.

Combination of more than one morphologic pattern may also be seen (Fig. 27.8).

TABLE 27.5: Classification of Odontogenic Tumours.

A. BENIGN

a) Epithelial origin

1. Ameloblastoma
2. Adenomatoid odontogenic tumour (Adeno-ameloblastoma)
3. Calcifying epithelial odontogenic tumour

b) Mesenchymal origin

1. Odontogenic myxoma
2. Odontogenic fibroma
3. Cementoma

c) Mixed epithelial-mesenchymal origin

1. Ameloblastic fibroma
2. Ameloblastic fibro-odontoma
3. Complex odontomas

B. MALIGNANT

a) Epithelial origin

1. Malignant ameloblastoma
2. Ameloblastic carcinoma

b) Mesenchymal origin

- Ameloblastic fibrosarcoma

Odontogenic Adenomatoid Tumour (Adeno-ameloblastoma)

This is a benign tumour seen more frequently in females in their 2nd decade of life. The tumour is commonly associated with an unerupted tooth and thus closely resembles dentigerous cyst radiologically. Unlike ameloblastoma, adenomatoid odontogenic tumour is not invasive nor does it recur after enucleation.

Microscopically, the lesion has extensive cyst formations. The wall of cyst contains scanty fibrous connective tissue in which are present characteristic tubule-like structures composed of epithelial cells and hence the name 'adenomatoid' (gland-like).

Calcifying Epithelial Odontogenic Tumour

This is a rare lesion which is locally invasive and recurrent like ameloblastoma. It is seen commonly in 4th and 5th decades and occurs more commonly in the region of mandible.

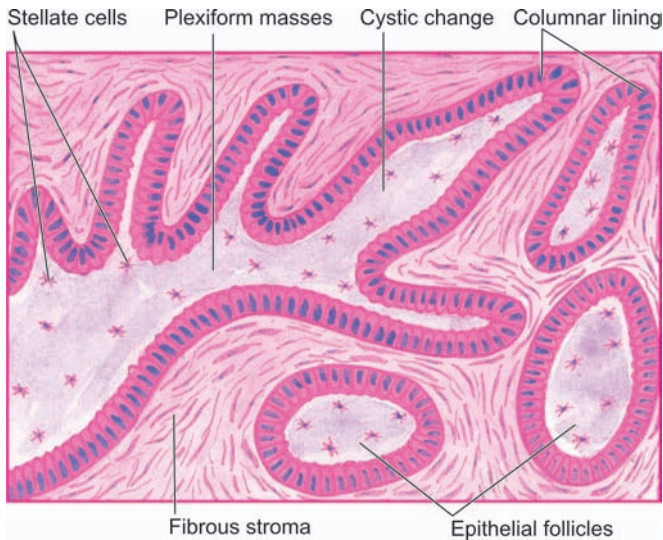


FIGURE 27.8

Ameloblastoma, follicular and plexiform patterns. Epithelial follicles are composed of central area of stellate cells and peripheral layer of cuboidal or columnar cells. Plexiform areas show irregular plexiform masses and network of strands of epithelial cells. A few areas show central cystic change.

Microscopically, the tumour consists of closely packed polyhedral epithelial cells having features of nuclear pleomorphism, giant nuclei and rare mitotic figures. The stroma is often scanty and appears homogeneous and hyalinised in which small calcified deposits are seen which are a striking feature of this tumour.

Odontogenic Myxoma (Myxofibroma)

Odontogenic myxoma is a locally invasive and recurring tumour.

Microscopically, it is characterised by abundant mucoid stroma and loose stellate cells in which are seen a few strands of odontogenic epithelium.

Ameloblastic Fibroma

This is a benign tumour consisting of epithelial and connective tissues derived from odontogenic apparatus. It resembles ameloblastoma but can be distinguished from it because ameloblastic fibroma occurs in younger age group (below 20 years) and the clinical behaviour is always benign.

Microscopically, it consists of epithelial follicles similar to those of ameloblastoma, set in a very cellular connective tissue stroma.

Odontomas

Odontomas are hamartomas that contain both epithelial and mesodermal dental tissue components. There are 3 subtypes:

i) **Complex odontoma** is always benign and consists of enamel, dentine and cementum which are not differentiated, so that the structure of actual tooth is not identifiable.

ii) **Compound odontoma** is also benign and is comprised of differentiated dental tissue elements forming a number of denticles in fibrous tissue.

iii) **Ameloblastic fibro-odontoma** is a lesion that resembles ameloblastic fibroma with odontoma formation.

Cementomas

Cementomas are a variety of benign lesions which are characterised by the presence of cementum or cementum-like tissue. Five types of cementomas are described:

i) **Benign cementoblastoma (true cementoma)** is a solitary lesion of jaw, characterised by features comparable to those of osteoid osteoma and osteoblastoma.

ii) **Cementifying fibroma** consists of cellular fibrous tissue containing calcified masses of cementum-like tissue.

iii) **Periapical cemental dysplasia (Periapical fibrous dysplasia)** is most common and resembles cementifying fibroma except that it contains more fibrous tissue as well as cementum-like tissue.

iv) **Multiple apical cementomas** are found on the apical region of teeth and detected incidentally in postmenopausal women.

v) **Gigantiform cementoma** is a large lobulated mass of cementum-like tissue. Sometimes, there are multiple such masses in the jaw.

B. MALIGNANT ODONTOGENIC TUMOURS

Malignant odontogenic tumours are rare.

Odontogenic Carcinoma

i) *Malignant ameloblastoma* is the term used for the uncommon metastasising ameloblastoma.

ii) *Ameloblastic carcinoma* is the term employed for the ameloblastic tumour having cytologic features of malignancy in the primary tumour.

iii) *Primary intraosseous carcinoma* may develop within the jaw from the rests of odontogenic epithelium.

iv) Rarely, carcinomas may arise from the odontogenic epithelium lining the *odontogenic cysts*.

Odontogenic Sarcomas

The only example of odontogenic sarcoma is a rare ameloblastic fibrosarcoma. This tumour resembles ameloblastic fibroma but in this the mesodermal compo-

nent is malignant (sarcomatous) whereas the ameloblastic epithelium remains differentiated and benign.

SALIVARY GLANDS

NORMAL STRUCTURE

There are two main groups of salivary glands—major and minor. The major salivary glands are the three paired glands: parotid, submandibular and sublingual. The minor salivary glands are numerous and widely distributed in the mucosa of oral cavity. The main duct of the parotid gland drains into the oral cavity opposite the second maxillary molar, while the ducts of submandibular and sublingual glands empty in the floor of the mouth. At times, heterotopic salivary gland tissue may be present in lymph nodes near or within the parotid gland.

Histologically, the salivary glands are tubuloalveolar glands and may contain mucous cells, serous cells, or both. The parotid gland is purely serous. The submandibular gland is mixed type but is predominantly serous, whereas the sublingual gland though also a mixed gland is predominantly mucous type. Similarly, minor salivary glands may also be serous, mucous or mixed type.

The secretory acini of the major salivary glands are drained by ducts lined by: low cuboidal epithelium in the intercalated portion, by tall columnar epithelium in the intralobular ducts, and by simpler epithelium in the secretory ducts.

The product of major salivary glands is *saliva* which performs various functions such as lubrication for swallowing and speech, and has enzyme amylase and antibacterial properties too.

SALIVARY FLOW DISTURBANCES

SIALORRHOEA (PTYALISM). Increased flow of saliva is termed sialorrhoea or ptyalism. It occurs commonly due to: stomatitis, teething, mentally retarded state, schizophrenia, neurological disturbances, increased gastric secretion and sialosis (i.e. uniform, symmetric, painless hypertrophy of salivary glands).

XEROSTOMIA. Decreased salivary flow is termed xerostomia. It is associated with the following conditions: Sjögren's syndrome, sarcoidosis, mumps parotitis, Mikulicz's syndrome, megaloblastic anaemia, dehydration, drug intake (e.g. antihistamines, antihypertensives, antidepressants).

SIALADENITIS

Inflammation of salivary glands, sialadenitis, may be acute or chronic, the latter being more common.

ETIOLOGY. Sialadenitis can occur due to the following causes:

1. Viral infections. The most common inflammatory lesion of the salivary glands particularly of the parotid glands, is mumps occurring in children of school-age. It is characterised by triad of pathological involvement—*epidemic parotitis (mumps)*, *orchitis-oophoritis*, and *pancreatitis* (Fig. 27.9). Involvement of testis and pancreas may lead to their atrophy. Less commonly, cytomegalovirus infection may occur in parotid glands of infants and young children.

2. Bacterial and mycotic infections. Bacterial infections may cause acute sialadenitis more often. Sometimes there are recurrent attacks of acute parotitis when parotitis becomes chronic.

i) **Acute sialadenitis.** The causes are:

- Acute infectious fevers
- Acute postoperative parotitis (ascent of microorganisms up the parotid duct from the mouth)
- General debility



FIGURE 27.9
Lesions in mumps.

- d) Old age
- e) Dehydration.
- ii) **Chronic sialadenitis.** This may result from the following causes:
 - a) *Recurrent obstructive type.* Recurrent obstruction due to calculi (sialolithiasis), stricture, surgery, injury etc may cause repeated attacks of acute sialadenitis by ascending infection and then chronicity.
 - b) *Recurrent non-obstructive type.* Recurrent mild ascending infection of the parotid gland may occur due to non-obstructive causes which reduce salivary secretion like due to intake of drugs causing hyposalivation (e.g. antihistamines, antihypertensives, antidepressants), effect of irradiation and congenital malformations of the duct system.
 - c) *Chronic inflammatory diseases.* Tuberculosis, actinomycosis and other mycoses may rarely occur in the salivary glands.
- 3. **Autoimmune disease.** Inflammatory changes are seen in salivary glands in 2 autoimmune diseases:
 - i) *Sjögren's syndrome* characterised by triad of dry eyes (keratoconjunctivitis sicca), dry mouth (xerostomia) and rheumatoid arthritis (Chapter 13).
 - ii) *Mikulicz's syndrome* is the combination of inflammatory enlargement of salivary and lacrimal glands with xerostomia.

PATHOLOGIC CHANGES. Irrespective of the underlying etiology of sialadenitis, there is swelling of the affected salivary gland, usually restricted by the fibrous capsule. Acute stage is generally associated with local redness, pain and tenderness with purulent ductal discharge. Late chronic cases may be replaced by firm fibrous swelling.

Microscopically, *acute viral sialadenitis* in mumps shows swelling and cytoplasmic vacuolation of the acinar epithelial cells and degenerative changes in the ductal epithelium. There is interstitial oedema, fibrinoid degeneration of the collagen and dense infiltration by mononuclear cells (lymphocytes, plasma cells and macrophages). *Chronic and recurrent sialadenitis* is characterised by increased lymphoid tissue in the interstitium, progressive loss of secretory tissue and replacement by fibrosis.

TUMOURS OF SALIVARY GLANDS

The major as well as minor salivary glands can give rise to a variety of benign and malignant tumours (Table 27.6). The major glands, particularly the parotid glands

TABLE 27.6: Classification of Salivary Gland Tumours.

A. BENIGN

1. Adenomas

- i) Pleomorphic adenoma (Mixed tumour) (65-80%)
- ii) Monomorphic adenoma
 - (a) Warthin's tumour (Papillary cystadenoma lymphomatosum, Adenolymphoma) (5-10%)
 - (b) Oxyphil adenoma (Oncocytomas) (< 1%)
 - (c) Other types (Myoepithelioma, Basal cell adenoma, Clear cell adenoma) (uncommon)

2. Mesenchymal tumours (rare)

B. MALIGNANT

- 1. Mucoepidermoid carcinoma (5-10%)
- 2. Malignant mixed tumour
 - i) Carcinoma in pleomorphic adenoma (2%)
 - ii) Carcinosarcoma (rare)
 - iii) Metastasising mixed salivary tumour (rare)
- 3. Adenoid cystic carcinoma (cylindroma) (3-10%)
- 4. Acinic cell carcinoma (2-3%)
- 5. Adenocarcinoma (1-3%)
- 6. Epidermoid carcinoma (1-3%)
- 7. Undifferentiated carcinoma (<1%)
- 8. Miscellaneous (rare)

(85%), are the most common sites. Majority of the parotid gland tumour (65-85%) are benign, while in the other major and minor salivary glands 35-50% of the tumours are malignant. Most of the salivary gland tumours originate from the ductal lining epithelium and the underlying myoepithelial cells; a few arise from acini. Recurrent tumours of the parotid glands, due to their location, are often associated with facial palsy and obvious scarring following surgical treatment.

A. BENIGN SALIVARY GLAND TUMOURS

ADENOMAS

The adenomas of the salivary glands are benign epithelial tumours. They are broadly classified into 2 major groups—pleomorphic and monomorphic adenomas.

Pleomorphic Adenoma (Mixed Salivary Tumour)

This is the most common tumour of major (60-75%) and minor (50%) salivary glands. Pleomorphic adenoma is the commonest tumour in the parotid gland and occurs less often in other major and minor salivary glands. The tumour is commoner in women and is seen more frequently in 3rd to 5th decades of life. The tumour is solitary, smooth-surfaced but sometimes nodular, painless and slow-growing. It is often located below and in front of the ear (Fig. 27.10).

PATHOLOGIC CHANGES. *Grossly,* pleomorphic adenoma is a circumscribed, pseudoencapsulated,

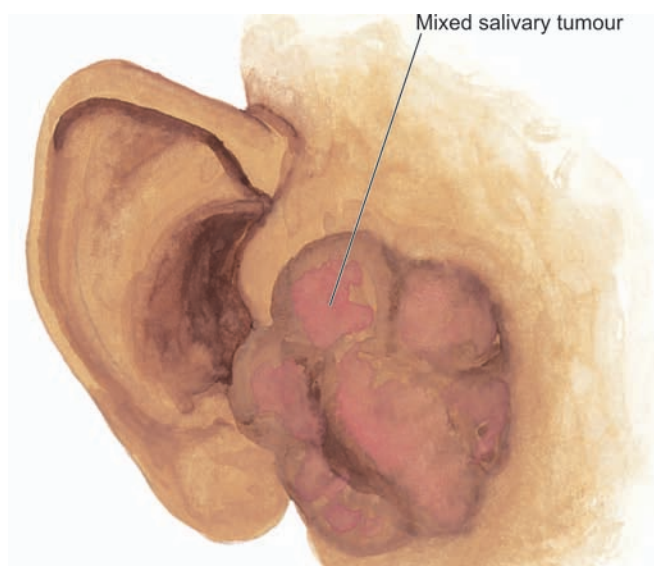


FIGURE 27.10

Pleomorphic adenoma (mixed salivary tumour) of the parotid gland.

rounded, at times multilobulated, firm mass, 2-5 cm in diameter, with bosselated surface. The cut surface is grey-white and bluish, variegated, semitranslucent, usually solid but occasionally may show small cystic spaces. The consistency is soft and mucoid.

Microscopically, the pleomorphic adenoma is characterised by pleomorphic or 'mixed' appearance in which there are epithelial elements present in a matrix of mucoid, myxoid and chondroid tissue (Fig. 27.11) (COLOUR PLATE IX: CL 36):

■ **The epithelial component** may form various patterns like ducts, acini, tubules, sheets and strands of cells of ductal or myoepithelial origin. The ductal cells are cuboidal or columnar, and the underlying myoepithelial cells may be polygonal or spindle-shaped resembling smooth muscle cells. The material found in the lumina of duct-like structures is PAS-positive epithelial mucin. Focal areas of squamous metaplasia and keratinisation may be present. Immunohistochemically, the tumour cells are immunoreactive for epithelial (cytokeratin, EMA, CEA) as well as myoepithelial (actin, vimentin) antibodies.

■ **The mesenchymal elements** are present as loose connective tissue and as myxoid, mucoid and chondroid matrix, which simulates cartilage (*pseudocartilage*) but is actually connective tissue mucin. More recently, the matrix of the tumour has been characterised as a product of myoepithelial cells. However, true cartilage and even bone is observed in a small proportion of these tumours.

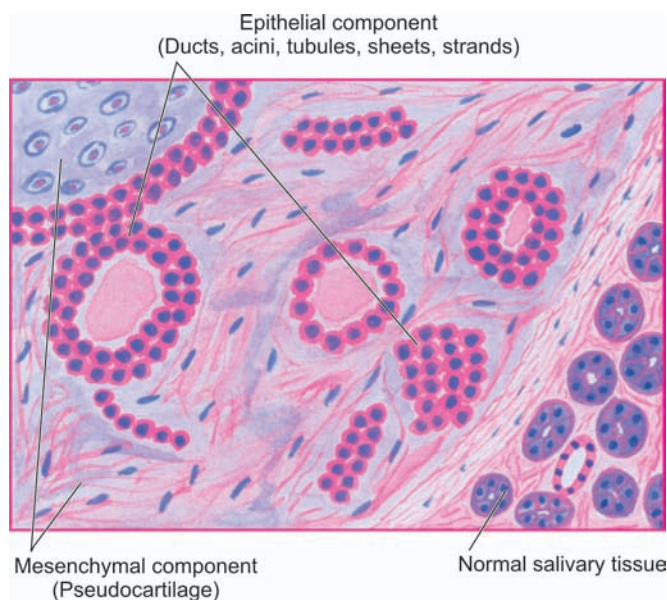


FIGURE 27.11

Pleomorphic adenoma, typical microscopic appearance. The epithelial element is comprised of ducts, acini, tubules, sheets and strands of cuboidal and myoepithelial cells. These are seen randomly admixed with mesenchymal elements composed of pseudocartilage which is the matrix of myxoid, chondroid and mucoid material.

The epithelial and mesenchymal elements are intermixed and either of the two components may be dominant in any tumour.

PROGNOSIS. Pleomorphic adenoma is notorious for recurrences, sometimes after many years. The main factors responsible for the tendency to recur are incomplete surgical removal due to proximity to the facial nerve, multiple foci of tumour, pseudoencapsulation, and implantation in the surgical field. Although the tumour is entirely benign, under exceptionally rare circumstances, an ordinary pleomorphic adenoma may metastasise to distant sites which too will have benign appearance as the original tumour. However, actual malignant transformation can also occur in a pleomorphic adenoma (*vide infra*).

Monomorphic Adenomas

These are benign epithelial tumours of salivary glands without any evidence of mesenchyme-like tissues. Their various forms are as under:

a) WARTHIN'S TUMOUR (PAPILLARY CYSTADENOMA LYMPHOMATOSUM, ADENOLYMPHOMA). It is a benign tumour of the parotid gland comprising about 8% of all parotid neoplasms, seen more commonly in men from 4th to 7th decades of life.

Rarely, it may arise in the submandibular gland or in minor salivary glands. *Histogenesis* of the tumour has been much debated. Currently, most accepted theory is that the tumour develops from parotid ductal epithelium present in lymph nodes adjacent to or within parotid gland.

PATHOLOGIC CHANGES. *Grossly*, the tumour is encapsulated, round or oval with smooth surface. The cut surface shows characteristic slit-like or cystic spaces, containing milky fluid and having papillary projections.

Microscopically, the tumour shows 2 components: epithelial parenchyma and lymphoid stroma (Fig. 27.12).

■ **The epithelial parenchyma** is composed of glandular and cystic structures having papillary arrangement and lined by characteristic eosinophilic epithelium. Variants of epithelial patterns include presence of mucous goblet cells and sebaceous differentiation.

■ **The lymphoid stroma** is present under the epithelium in the form of prominent lymphoid tissue, often with germinal centres.

b) OXYPHIL ADENOMA (ONCOCYTOMA). It is a benign slow-growing tumour of the major salivary glands. The tumour consists of parallel sheets, acini or tubules of large cells with glandular eosinophilic cytoplasm (oncocytes) and hence the name.

c) OTHER TYPES OF MONOMORPHIC ADENOMAS. There are some uncommon forms of monomorphic adenomas:

- i) *Myoepithelioma* is an adenoma composed exclusively of myoepithelial cells which may be arranged in tubular, alveolar or trabecular pattern.
- ii) *Basal cell adenoma* is characterised by the type and arrangement of cells resembling basal cell carcinoma of the skin.
- iii) *Clear cell adenoma* has spindle-shaped or polyhedral cells with clear cytoplasm.

MISCELLANEOUS BENIGN TUMOURS

A number of mesenchymal tumours can rarely occur in salivary glands. These include: fibroma, lipoma, neurilemmomas, neurofibroma, haemangioma and lymphangioma.

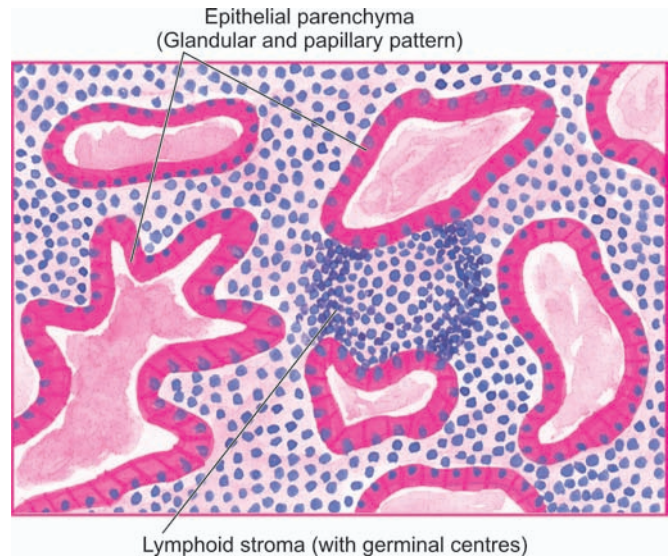


FIGURE 27.12

Warthin's tumour, showing eosinophilic epithelium forming glandular and papillary, cystic pattern with intervening stroma of lymphoid tissue.

B. MALIGNANT SALIVARY GLAND TUMOURS

Mucoepidermoid Carcinoma

The status of 'mucoepidermoid tumour' as an intermediate grade tumour in the previous classification has undergone upgradation to full-fledged mucoepidermoid carcinoma now having the following peculiar features:

- It is the most *common malignant* salivary gland tumour (both in the major and minor glands).
- The *parotid gland* amongst the major salivary glands and the minor salivary glands in the *palate* are the most common sites.
- The common age group affected is 30-60 years but it is also the most common malignant salivary gland tumour affecting *children and adolescents*.
- It is the most common example of *radiation-induced* malignant tumour, especially therapeutic radiation.

PATHOLOGIC CHANGES. *Grossly*, the tumour is usually circumscribed but not encapsulated. It varies in size from 1 to 4 cm.

Microscopically, the tumour is classified into low, intermediate and high grade depending upon the degree of differentiation and tumour invasiveness. The tumour is composed of combination of epidermoid cells and mucus-secreting cells (as the name implies) as also cells with intermediate differentiation between these two cell types and clear cells.

Malignant Mixed Tumour

Malignant mixed tumour comprises three distinct clinicopathologic entities:

- Carcinoma arising in benign mixed salivary gland tumour (carcinoma *ex* pleomorphic adenoma);
- Carcinosarcoma; and
- Metastasising mixed salivary tumour.

Carcinoma *ex* pleomorphic adenoma is more common while the other two are rare tumours. Approximately 2 to 5% of pleomorphic adenomas reveal areas of frank malignancy. The slow-growing adenoma may have been present for a number of years when suddenly it undergoes rapid increase in its size, becomes painful and the individual may develop facial palsy. Malignant transformation occurs in later age (6th decade) than the usual age for pleomorphic adenoma (4th to 6th decades). It may occur in primary tumour but more often occurs in its recurrences.

PATHOLOGIC CHANGES. *Grossly*, the tumour is poorly-circumscribed with irregular infiltrating margin. Cut section may show haemorrhages, necrosis and cystic degeneration.

Microscopically, besides the typical appearance of pleomorphic adenoma, malignant areas show cytologic features of carcinoma such as anaplasia, nuclear hyperchromatism, large nucleolisation, mitoses and evidence of invasive growth. All types of usual salivary gland carcinomas (described below) may develop in pleomorphic adenoma.

Adenoid Cystic Carcinoma (Cylindroma)

This is a highly malignant tumour due to its typical infiltrative nature, especially along the nerve sheaths. Adenoid cystic carcinoma is histologically characterised by cribriform appearance i.e. the epithelial tumour cells of duct-lining and myoepithelial cells are arranged in

duct-like structures or masses of cells, having typical fenestrations or cyst-like spaces and hence the name 'adenoid cystic'.

Acinic Cell Carcinoma

This is a rare tumour composed of acinic cells resembling serous cells of normal salivary gland. These cells are arranged in sheets or acini and have characteristic basophilic granular cytoplasm. The degree of atypia may vary from a benign cytologic appearance to cellular features of malignancy.

Adenocarcinoma

Adenocarcinoma of the salivary gland does not differ from adenocarcinoma elsewhere in the body. It may have some variants such as mucoid adenocarcinoma, clear-cell adenocarcinoma and papillary cystadenocarcinoma.

Epidermoid Carcinoma

This rare tumour has features of squamous cell carcinoma with keratin formation and has intercellular bridges. The tumour commonly infiltrates the skin and involves the facial nerve early.

Undifferentiated Carcinoma

This highly malignant tumour consists of anaplastic epithelial cells which are too poorly differentiated to be placed in any other known category.

Miscellaneous Malignant Tumours

Some rare malignant tumour of epithelial and mesenchymal origin are melanoma, sebaceous carcinoma, undifferentiated carcinoma, lymphoma, fibrosarcoma and leiomyosarcoma and are similar in morphology to such tumours elsewhere in the body. Besides, metastatic involvement of major salivary glands or the adjacent lymph nodes is common, especially from epidermoid carcinoma and malignant melanoma.



Jaundice, Viral Hepatitis, Cirrhosis

JAUNDICE

Jaundice or icterus refers to the yellow pigmentation of the skin or sclerae by bilirubin. Bilirubin pigment has high affinity for elastic tissue and hence jaundice is particularly noticeable in tissues rich in elastin content. Jaundice is the result of elevated levels of bilirubin in the blood termed hyperbilirubinaemia. Normal serum bilirubin concentration ranges from 0.2-0.8 mg/dl, about 80% of which is unconjugated. Jaundice becomes clinically evident when the total serum bilirubin exceeds 2 mg/dl. A rise of serum bilirubin between the normal and 2 mg/dl is generally not accompanied by visible jaundice and is called *latent jaundice*.

Before considering the features and types of jaundice, it is essential to review the normal bilirubin metabolism.

NORMAL BILIRUBIN METABOLISM

Normal metabolism of bilirubin can be conveniently described under 4 main headings—source, transport, hepatic phase and intestinal phase as illustrated schematically in Fig. 28.1.

1. SOURCE OF BILIRUBIN. About 80-85% of the bilirubin is derived from the catabolism of haemoglobin present in senescent red blood cells. The destruction of effete erythrocytes at the end of their normal life-span of 120 days takes place in the reticuloendothelial system in the bone marrow, spleen and liver. The remaining 15-20% of the bilirubin comes partly from non-haemoglobin haem-containing pigments such as myoglobin, catalase and cytochromes, and partly from ineffective erythropoiesis. In either case, haem moiety is formed which is converted to biliverdin by microsomal haem oxygenase for which oxygen and NADPH are essential requirements. Bilirubin is formed from biliverdin by biliverdin reductase.

2. TRANSPORT OF BILIRUBIN. Bilirubin on release from macrophages circulates as unconjugated bilirubin in plasma tightly bound to albumin. Certain drugs such as sulfonamides and salicylates compete with bilirubin for albumin binding and displace bilirubin from albumin, thus facilitating bilirubin to enter into the brain

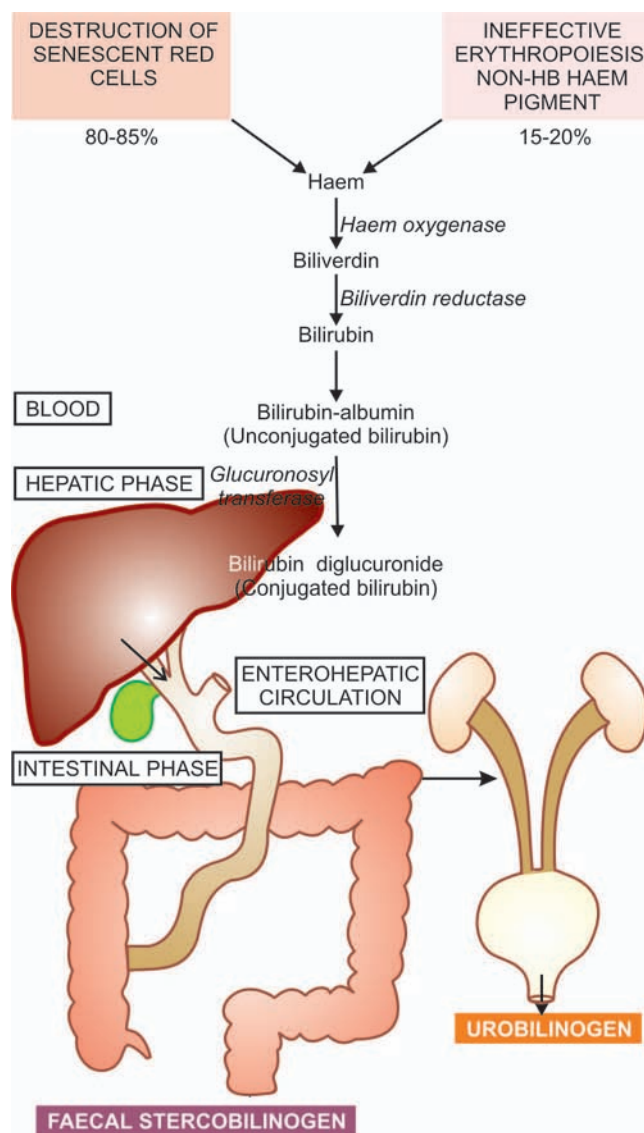


FIGURE 28.1

Normal red cell destruction in the RE system.

in neonates and increase the risk of *kernicterus*. Bilirubin is found in body fluids in proportion to their albumin content such as in CSF, joint effusions, cysts etc.

3. HEPATIC PHASE. On coming in contact with the hepatocyte surface, unconjugated bilirubin is prefe-

rentially metabolised which involves 3 steps: hepatic uptake, conjugation and secretion in bile.

i) Hepatic uptake: Albumin-bound unconjugated bilirubin upon entry into the hepatocyte, is dissociated into bilirubin and albumin. The bilirubin gets bound to cytoplasmic protein *glutathione-S-transferase (GST)* (earlier called ligandin).

ii) Conjugation: Unconjugated bilirubin is not water-soluble but is alcohol-soluble and is converted into water-soluble compound by conjugation. Conjugation occurs in endoplasmic reticulum and involves conversion to bilirubin diglucuronide by the action of microsomal enzyme, *bilirubin-UDP-glucuronosyl transferase*, to mono- and diglucuronides (Fig. 28.2). The process of conjugation can be induced by drugs like phenobarbital.

Conjugated bilirubin is bound to albumin in two forms: reversible and irreversible. Reversible binding is similar to that of unconjugated bilirubin. However, when present in serum for a long time (e.g. in cholestasis, long-standing biliary obstruction, chronic active hepatitis), conjugated bilirubin is bound to albumin irreversibly and is termed *delta bilirubin* or *bilioprotein*. This irreversible conjugated delta bilirubin is not excreted by the kidney, and remains detectable in serum for sufficient time after recovery from the diseases listed above.

iii) Secretion into bile: Conjugated (water soluble) bilirubin is rapidly transported directly into bile canaliculi by energy-dependent process and then excreted into the bile.

4. INTESTINAL PHASE. Appearance of conjugated bilirubin in the intestinal lumen is followed by either direct excretion in the stool as stercobilinogen which imparts the normal yellow colour to stool, or may be metabolised to urobilinogen by the action of intestinal bacteria. Conjugated bilirubin is normally not reabsorbable whereas its metabolic product, urobilinogen, is reabsorbed from the small intestine and reaches enterohepatic circulation. Some of the absorbed urobilinogen is resecreted by the liver into the bile while the rest is excreted in the urine as urobilinogen.

The major differences between unconjugated and conjugated bilirubin are summarised in Table 28.1.

CLASSIFICATION AND FEATURES OF JAUNDICE

Based on pathophysiology, jaundice may result from one or more of the following mechanisms:

1. Increased bilirubin production
2. Decreased hepatic uptake
3. Decreased hepatic conjugation

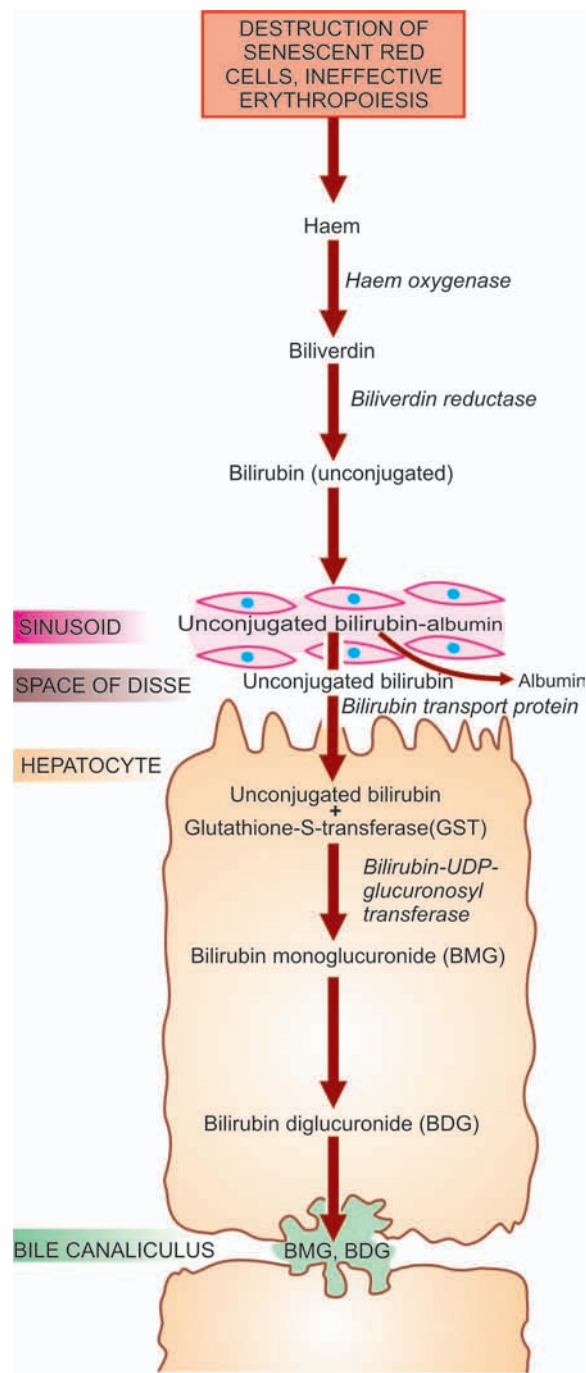


FIGURE 28.2

Schematic representation of hepatic phase of bilirubin transport.

4. Decreased excretion of bilirubin into bile

Accordingly, a simple classification of jaundice is to divide it into 3 predominant types: *pre-hepatic (haemolytic)*, *hepatic*, and *post-hepatic cholestatic*. Hyperbilirubinaemia due to first three mechanisms is *mainly unconjugated* while the last variety yields *mainly conjugated*

TABLE 28.1: Major Differences between Unconjugated and Conjugated Bilirubin.

FEATURE	UNCONJUGATED BILIRUBIN	CONJUGATED BILIRUBIN
1. <i>Normal serum level</i>	More	Less (less than 0.25 mg/dl)
2. <i>Water solubility</i>	Absent	Present
3. <i>Affinity to lipids (alcohol solubility)</i>	Present	Absent
4. <i>Serum albumin binding</i>	High	Low
5. <i>van den Bergh reaction</i>	Indirect (Total minus direct)	Direct
6. <i>Renal excretion</i>	Absent	Present
7. <i>Bilirubin albumin covalent complex formation</i>	Absent	Present
8. <i>Affinity to brain tissue</i>	Present (Kernicterus)	Absent

hyperbilirubinaemia. A simple test to determine whether hyperbilirubinaemia is of unconjugated or conjugated variety is to determine whether bilirubin is present in urine or not; its absence in urine suggests unconjugated hyperbilirubinaemia since unconjugated bilirubin is not filtered by the glomerulus. The presence of bilirubin in the urine is evidence of conjugated hyperbilirubinaemia.

Based on these mechanisms, the pathogenesis and main features of the two predominant forms of hyperbilirubinaemia are discussed below (Table 28.2).

Predominantly Unconjugated Hyperbilirubinaemia

This form of jaundice can result from the following three sets of conditions:

1. INCREASED BILIRUBIN PRODUCTION (HAEMOLYTIC, ACHOLURIC OR PREHEPATIC JAUNDICE). This results from excessive red cell destruction as occurs in intra- and extravascular haemolysis or due to ineffective erythropoiesis. There is increased release of haemoglobin from excessive breakdown of red cells that leads to overproduction of bilirubin. Hyperbilirubinaemia develops when the capacity of the liver to conjugate large amount of bilirubin is exceeded. In premature infants, the liver is deficient in enzyme necessary for conjugation while the rate of red cell destruction is high. This results in *icterus neonatorum* which is particularly severe in haemolytic disease of the newborn due to maternal isoantibodies (page 516). Since there is predominantly unconjugated hyperbilirubinaemia in such cases, there is danger of permanent brain damage in these infants from kernicterus when the serum level of unconjugated bilirubin exceeds 20 mg/dl.

Laboratory data in haemolytic jaundice, in addition to predominant unconjugated hyperbilirubinaemia, reveal normal serum levels of transaminases, alkaline phosphatase and proteins. Bile pigment being

TABLE 28.2: Pathophysiologic Classification of Jaundice.

I. PREDOMINANTLY UNCONJUGATED HYPERBILIRUBINAEMIA

1. Increased bilirubin production (Haemolytic, acholuric or prehepatic jaundice)

- Intra- and extravascular haemolysis
- Ineffective erythropoiesis

2. Decreased hepatic uptake

- Drugs
- Prolonged starvation
- Sepsis

3. Decreased bilirubin conjugation

- Hereditary disorders (e.g. Gilbert's syndrome, Crigler-Najjar syndrome)
- Acquired defects (e.g. drugs, hepatitis, cirrhosis)
- Neonatal jaundice

II. PREDOMINANTLY CONJUGATED HYPERBILIRUBINAEMIA (CHOLESTASIS)

1. Intrahepatic cholestasis (Impaired hepatic excretion)

- Hereditary disorders or 'pure cholestasis' (e.g. Dubin-Johnson syndrome, Rotor's syndrome, fibrocystic disease of pancreas, benign familial recurrent cholestasis, intrahepatic atresia, cholestatic jaundice of pregnancy)
- Acquired disorders or 'hepatocellular cholestasis' (e.g. viral hepatitis, drugs, alcohol-induced injury, sepsis, cirrhosis)

2. Extrahepatic cholestasis (Extrahepatic biliary obstruction)

- Mechanical obstruction (e.g. gallstones, inflammatory strictures, carcinoma head of pancreas, tumours of bile ducts, sclerosing cholangitis, congenital atresia of extrahepatic ducts)

unconjugated type is absent from urine (acholuric jaundice). However, there is dark brown colour of stools due to excessive faecal excretion of bile pigment and increased urinary excretion of urobilinogen.

2. DECREASED HEPATIC UPTAKE. The uptake of bilirubin by the hepatocyte that involves dissociation of the pigment from albumin and its binding to cytoplasmic protein, GST or ligandin, may be deranged in certain conditions e.g. due to drugs, prolonged starvation and sepsis.

3. DECREASED BILIRUBIN CONJUGATION. This mechanism involves deranged hepatic conjugation due to defect or deficiency of the enzyme, glucuronosyl transferase. This can occur in certain inherited disorders of the enzyme (e.g. Gilbert's syndrome and Crigler-Najjar syndrome), or acquired defects in its activity (e.g. due to drugs, hepatitis, cirrhosis). However, hepatocellular damage causes deranged excretory capacity of the liver more than its conjugating capacity (*see below*). The physiologic neonatal jaundice is also partly due to relative deficiency of UDP-glucuronosyl transferase in the neonatal liver and is partly as a result of increased rate of red cell destruction in neonates.

II. Predominantly Conjugated Hyperbilirubinaemia (Cholestasis)

This form of hyperbilirubinaemia is defined as failure of normal amounts of bile to reach the duodenum. Morphologically, cholestasis means accumulation of bile in liver cells and biliary passages. The defect in excretion may be within the biliary canaliculi of the hepatocyte and in the microscopic bile ducts (*intrahepatic cholestasis or medical jaundice*), or there may be mechanical obstruction to the extrahepatic biliary excretory apparatus (*extrahepatic cholestasis or obstructive jaundice*). It is important to distinguish these two forms of cholestasis since extrahepatic cholestasis or obstructive jaundice is often treatable with surgery, whereas the intrahepatic cholestasis or medical jaundice cannot be benefitted by surgery but may in fact worsen by the operation. Prolonged cholestasis of either of the two types may progress to biliary cirrhosis (page 372).

1. INTRAHEPATIC CHOLESTASIS. Intrahepatic cholestasis is due to impaired hepatic excretion of bile and may occur from hereditary or acquired disorders.

i) Hereditary disorders producing intrahepatic obstruction to biliary excretion are characterised by 'pure cholestasis' e.g. in Dubin-Johnson syndrome, Rotor syndrome, fibrocystic disease of pancreas, benign familial

recurrent cholestasis, intrahepatic atresia and cholestatic jaundice of pregnancy.

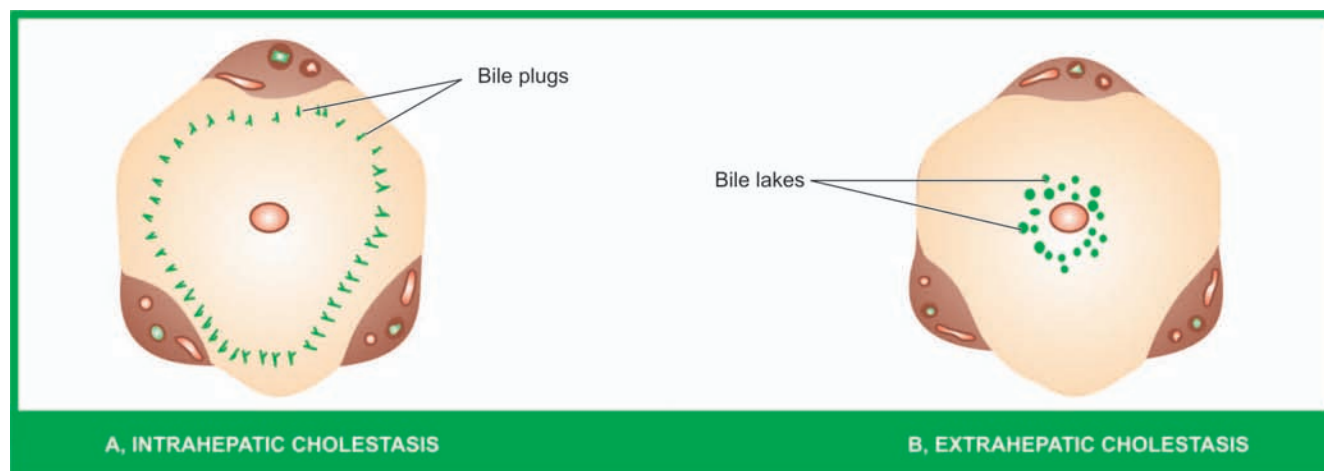
ii) Acquired disorders with intrahepatic excretory defect of bilirubin are largely due to hepatocellular diseases and hence are termed '*hepatocellular cholestasis*' e.g. in viral hepatitis, alcoholic hepatitis, and drug-induced cholestasis such as from administration of chlorpromazine and oral contraceptives.

The **features** of intrahepatic cholestasis include: predominant conjugated hyperbilirubinaemia due to regurgitation of conjugated bilirubin into blood, bilirubinuria, elevated levels of serum bile acids and consequent pruritus, elevated serum alkaline phosphatase, hyperlipidaemia and hypoprothrombinaemia. 'Pure cholestasis' can be distinguished from 'hepatocellular cholestasis' by elevated serum levels of transaminases in the latter due to liver cell injury.

■ **Liver biopsy** in cases with intrahepatic cholestasis reveals milder degree of cholestasis than the extrahepatic disorders (Fig. 28.3,A). The biliary canaliculi of the hepatocytes are dilated and contain characteristic elongated green-brown *bile plugs*. The cytoplasm of the affected hepatocytes shows feathery degeneration. Canalicular bile stasis eventually causes proliferation of intralobular ductules followed by periportal fibrosis and produces a picture resembling biliary cirrhosis.

2. EXTRAHEPATIC CHOLESTASIS. Extrahepatic cholestasis results from mechanical obstruction to large bile ducts outside the liver or within the porta hepatis. The common causes are gallstones, inflammatory strictures, carcinoma head of pancreas, tumours of bile duct, sclerosing cholangitis and congenital atresia of extrahepatic ducts. The obstruction may be complete and sudden with eventual progressive obstructive jaundice, or the obstruction may be partial and incomplete resulting in intermittent jaundice.

The **features** of extrahepatic cholestasis (obstructive jaundice), like in intrahepatic cholestasis, are predominant conjugated hyperbilirubinaemia, bilirubinuria, elevated serum bile acids causing intense pruritus, high serum alkaline phosphatase and hyperlipidaemia. However, there are certain features which help to distinguish extrahepatic from intrahepatic cholestasis. In obstructive jaundice, there is malabsorption of fat-soluble vitamins (A,D,E and K) and steatorrhea resulting in vitamin K deficiency. Prolonged prothrombin time in such cases shows improvement following parenteral administration of vitamin K, whereas hypoprothrombinaemia due to hepatocellular disease shows no such improvement in prothrombin time with vitamin

**FIGURE 28.3**

Salient features in morphology of liver in intra- and extrahepatic cholestasis. A, Intrahepatic cholestasis is characterised by elongated bile plugs in the canaliculi of hepatocytes at the periphery of the lobule. B, Extrahepatic cholestasis shows characteristic bile lakes due to rupture of canaliculi in the hepatocytes in the centrilobular area.

K administration. The stools of such patients are clay-coloured due to absence of bilirubin metabolite, stercobilin, in faeces and there is virtual disappearance of urobilinogen from the urine. These patients may have fever due to high incidence of ascending bacterial infections (ascending cholangitis).

■ **Liver biopsy** in cases with extrahepatic cholestasis shows more marked changes of cholestasis (Fig. 28.3,B). Since the obstruction is in the extrahepatic bile ducts, there is progressive retrograde extension of bile stasis into intrahepatic duct system. This results in dilatation of bile ducts and rupture of canaliculi with extravasation of bile producing *bile lakes*. Since bile is toxic, the regions of bile lakes are surrounded by focal necrosis of hepatocytes. Stasis of bile predisposes to ascending bacterial infections with accumulation of polymorphs around the dilated ducts (ascending cholangitis). Eventually, there is proliferation of bile ducts and the appearance may mimic biliary cirrhosis.

NEONATAL JAUNDICE

Jaundice appears in neonates when the total serum bilirubin is more than 3 mg/dl. It may be the result of unconjugated or conjugated hyperbilirubinaemia; the former being more common. Important causes of neonatal jaundice are listed in Table 28.3.

Hereditary non-haemolytic hyperbilirubinaemias are a small group of uncommon familial disorders of bilirubin metabolism when haemolytic causes have been excluded. The commonest is Gilbert's syndrome; others

are Crigler-Najjar syndrome, Dubin-Johnson syndrome, Rotor's syndrome and benign familial recurrent cholestasis. The features common to all these conditions are presence of icterus but almost normal liver function tests and no well-defined morphologic changes except in Dubin-Johnson syndrome. Gilbert's syndrome and Crigler-Najjar syndrome are examples of *hereditary non-haemolytic unconjugated hyperbilirubinaemia*, whereas Dubin-Johnson syndrome, Rotor's syndrome and benign familial recurrent cholestasis are conditions with *hereditary conjugated hyperbilirubinaemia*.

TABLE 28.3: Causes of Neonatal Jaundice.

A. Unconjugated hyperbilirubinaemia

1. Physiologic and prematurity jaundice
2. Haemolytic disease of the newborn and kernicterus (page 516)
3. Congenital haemolytic disorders (page 468)
4. Perinatal complications (e.g. haemorrhage, sepsis)
5. Gilbert's syndrome
6. Crigler-Najjar syndrome (type I and II)

B. Conjugated hyperbilirubinaemia

1. Hereditary (Dubin-Johnson syndrome, Rotor's syndrome)
2. Infections (e.g. hepatitis B, hepatitis C or non-A non-B hepatitis, rubella, coxsackievirus, cytomegalovirus, echovirus, herpes simplex, syphilis, toxoplasma, gram-negative sepsis)
3. Metabolic (e.g. galactosaemia, alpha-1-antitrypsin deficiency, cystic fibrosis, Niemann-Pick disease)
4. Idiopathic (neonatal hepatitis, congenital hepatic fibrosis)
5. Biliary atresia (intrahepatic and extrahepatic)
6. Reye's syndrome

LIVER CELL NECROSIS

All forms of injury to the liver such as microbiologic, toxic, circulatory or traumatic, result in necrosis of liver cells. The extent of involvement of hepatic lobule in necrosis varies. Accordingly, liver cell necrosis is divided into 3 types: *diffuse* (submassive to massive), *zonal* and *focal*.

1. DIFFUSE (SUBMASSIVE TO MASSIVE) NECROSIS. When there is extensive and diffuse necrosis of the liver involving all the cells in groups of lobules, it is termed diffuse, or submassive to massive necrosis. It is most commonly caused by viral hepatitis or drug toxicity.

2. ZONAL NECROSIS. Zonal necrosis is necrosis of hepatocytes in 3 different zones of the hepatic lobule (Fig. 28.4). Accordingly, it is of 3 types; each type affecting respective zone is caused by different etiologic factors:

i) Centrilobular necrosis is the commonest type involving hepatocytes in zone 3 (i.e. located around the central vein). Centrilobular necrosis is characteristic feature of ischaemic injury such as in shock and CHF since zone 3 is farthest from the blood supply. Besides, it also occurs in poisoning with chloroform, carbon tetrachloride and certain drugs.

ii) Midzonal necrosis is uncommon and involves zone 2 of the hepatic lobule. This pattern of necrosis is seen

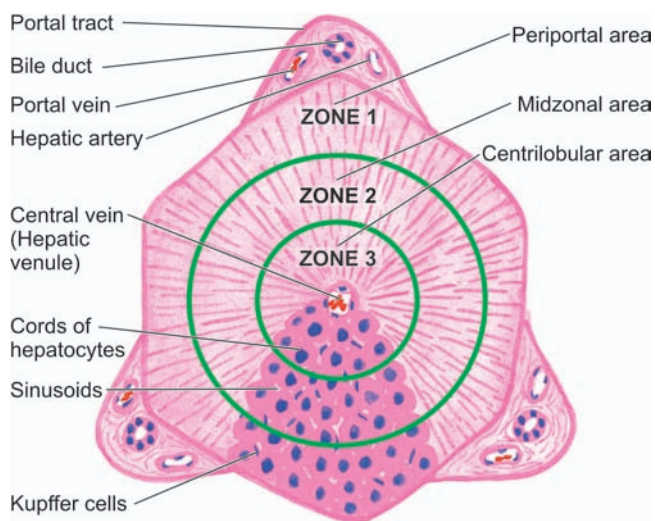


FIGURE 28.4

Histology of hepatic lobule. The hexagonal or pyramidal structure with central vein and peripheral 4 to 5 portal triads is termed the classical lobule. The functional divisions of the lobule into 3 zones are shown by circles.

in yellow fever and viral hepatitis. In viral hepatitis, some of the necrosed hepatocytes of the mid-zone are transformed into acidophilic, rounded Councilman bodies.

iii) Periportal (peripheral) necrosis is seen in zone 1 involving the parenchyma closest to the arterial and portal blood supply. Since zone 1 is most well perfused, it is most vulnerable to the effects of circulating hepatotoxins e.g. in phosphorus poisoning and eclampsia.

3. FOCAL NECROSIS. This form of necrosis involves small groups of hepatocytes irregularly distributed in the hepatic lobule. Focal necrosis is most often caused by microbiologic infections. These include viral hepatitis, miliary tuberculosis, typhoid fever and various other forms of bacterial, viral and fungal infections. Focal necrosis may also occur in drug-induced hepatitis.

VIRAL HEPATITIS

The term viral hepatitis is used to describe infection of the liver caused by hepatotropic viruses. Currently there are 5 main varieties of these viruses and a sixth poorly-characterised virus, causing distinct types of viral hepatitis:

- *Hepatitis A virus (HAV)*, causing a faecally-spread self-limiting disease;
- *Hepatitis B virus (HBV)*, causing a parenterally transmitted disease that may become chronic;
- *Hepatitis C virus (HCV)*, previously termed non-A, non-B (NANB) hepatitis virus involved chiefly in transfusion-related hepatitis;
- *Hepatitis delta virus (HDV)* which is sometimes associated as superinfection with hepatitis B infection;
- *Hepatitis E virus (HEV)*, causing water-borne infection; and
- *Hepatitis G virus (HGV)*, is a recently discovered parenterally transmitted hepatotropic virus.

All these human hepatitis viruses are RNA viruses except HBV which is a DNA virus.

Though a number of other viral diseases such as infection with Epstein-Barr virus (in infectious mononucleosis), arbovirus (in yellow fever), cytomegalovirus, herpes simplex and several others affect the liver but the changes produced by them are nonspecific and the term 'viral hepatitis' is strictly applied to infection of the liver by the hepatitis viruses.

ETIOLOGIC CLASSIFICATION

Based on the etiologic agent, viral hepatitis is currently classified into 6 etiologic types—hepatitis A, hepatitis

B, hepatitis C, hepatitis D, hepatitis E and hepatitis G. The contrasting features of various types are presented in Table 28.4.

Hepatitis A

Infection with HAV causes hepatitis A (infectious hepatitis). Hepatitis A is responsible for 20-25% of clinical hepatitis in the developing countries of the world but the incidence is much lower in the developed countries. Hepatitis A is usually a benign, self-limiting disease and has an incubation period of 15-45 days. The disease occurs in epidemic form as well as sporadically. It is usually spread by faeco-oral route. Parenteral transmission is extremely rare. The spread is related to close personal contact such as in overcrowding, poor hygiene and poor sanitation. Most frequently affected age is 5-14 years; adults are often infected by spread from children.

HEPATITIS A VIRUS (HAV). The etiologic agent for hepatitis A, HAV, is a small, 27 nm diameter, icosahedral non-enveloped, single-stranded RNA virus. Viral genome has been characterised but only a single serotype has been identified. HAV infection can be transmitted to primates and the virus can be cultivated *in vitro*. Inactivation of viral activity can be achieved by boiling for 1 minute, by ultraviolet radiation or by contact with formaldehyde and chlorine. The virus is present in the liver cells, bile, stool and blood during the incubation period and in pre-icteric phase but viral shedding diminishes after the onset of jaundice. Chronic carriers have not been identified for HAV infection.

PATHOGENESIS. The mechanism by which HAV infection causes hepatitis A is poorly understood. An immunologic basis is suspected but the evidence in support is indirect in the form of immunologic markers

TABLE 28.4: Features of Various Types of Hepatitis Viruses.

FEATURE	HEPATITIS A	HEPATITIS B	HEPATITIS C	HEPATITIS D	HEPATITIS E	HEPATITIS G
1. <i>Agent</i>	HAV	HBV	HCV	HDV	HEV	HGV
2. <i>Year identified</i>	1973	1965	1989	1977	1980	1995
3. <i>Viral particle</i>	27 nm	42 nm	30-60 nm	35-37 nm	32-34 nm	?
4. <i>Genome</i>	RNA, ss, linear	DNA, ss/ds	RNA, ss, linear circular	RNA, ss, circular	RNA, ss, linear	RNA, ss, linear
5. <i>Morphology</i>	Icosahedral non-enveloped	Double-shelled, enveloped	Enveloped	Enveloped, replication defective	Icosahedral, non-enveloped	?
6. <i>Spread</i>	Faeco-oral	Parenteral, close contact	Parenteral, close contact	Parenteral, close contact	Water-borne	Parenteral
7. <i>Incubation period</i>	15-45 days	30-180 days	20-90 days	30-50 days (In superinfection)	15-60 days	?
8. <i>Antigen(s)</i>	HAV	HBsAg HBcAg HBeAg HBxAg	HCV RNA C 100-3 C 33c NS5	HBsAg HDV	HEV	?
9. <i>Antibodies</i>	anti-HAV	anti-HBs anti-HBc anti-HBe	anti-HCV	anti-HBs anti-HDV	anti-HEV	?
10. <i>Severity</i>	Mild	Occasionally severe	Moderate	Occasionally severe	Mild	?
11. <i>Chronic hepatitis</i>	None	Occasional	Common	Common	None	?
12. <i>Carrier state</i>	None	<1%	<1%	1-10%	Unknown	1-2%
13. <i>Hepatocellular carcinoma</i>	No	+	+	±	None	?
14. <i>Prognosis</i>	Excellent	Worse with age	Moderate	Acute good; chronic poor	Good	?

ss= single-stranded; ss/ds= partially single-stranded partially double-stranded.

but not direct demonstration of the etiologic agent in the affected hepatocytes. These markers are (Fig. 28.5):

1. *IgM anti-HAV antibody* appears in the serum at the onset of symptoms of acute hepatitis A.
2. *IgG anti-HAV antibody* is detected in the serum after IgM antibody and gives life-long protective immunity against reinfection with HAV.

Hepatitis B

Hepatitis B (serum hepatitis) caused by HBV infection has a longer incubation period (30-180 days) and is transmitted parenterally such as in recipients of blood and blood products, intravenous drug addicts, patients treated by renal dialysis and hospital workers exposed to blood, and by intimate physical contact such as from mother to child and sexually. The disease may occur at any age. HBV infection causes more severe form of illness that includes: acute hepatitis B, chronic hepatitis, progression to cirrhosis, fulminant hepatitis and an asymptomatic carrier stage. HBV plays some role in the development of hepatocellular carcinoma as discussed later.

HEPATITIS B VIRUS (HBV). The etiologic agent for hepatitis B, HBV, is a DNA virus which has been extensively studied. Electron microscopic studies on serum of patients infected with HBV show 3 forms of viral particles of 2 sizes—small (spheres and tubules/filaments) and large (spheres) as under:

- i) *Small particles* are most numerous and are in two forms: as 22 nm spheres, and as tubules 22 nm in diameter and 100 nm long. These are antigenically identical to envelope protein of HBV and represent excess of viral envelope protein referred as hepatitis B surface antigen (HBsAg).

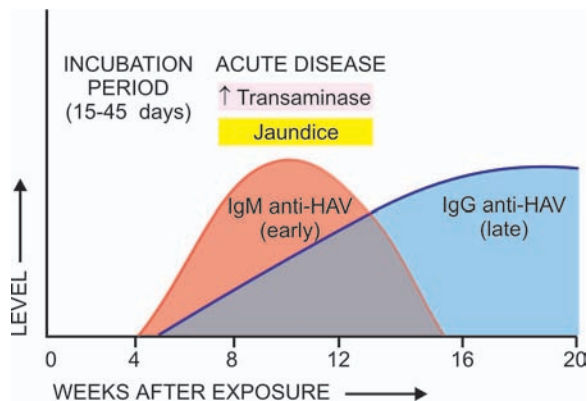


FIGURE 28.5

Sequence of appearance of antibodies to HAV.

- ii) *Large particles*, 42 nm in diameter, are double-shelled spherical particles, also called as *Dane particles*. These are about 100 to 1000 times less in number in serum compared to small 22 nm particles and represent intact virion of HBV.

The genomic structure of HBV is quite compact and complex. The HBV DNA consists of 4 overlapping genes which encode for multiple proteins (Fig. 28.6):

1. *S gene* codes for the major surface envelope protein, hepatitis B surface antigen (HBsAg). HBsAg is present on the outer surface of the large spherical particles as well as in small spherical and tubular structures. *Pre-S1* and *pre-S2* regions of genome which are upstream of S gene, code for two other large proteins.
2. *P gene* is the largest and codes for DNA polymerase.
3. *C gene* codes for two nucleocapsid proteins, HBeAg and a core protein termed HBcAg.
4. *X gene* codes for HBxAg having a role in activation and transcription of viral and cellular genes and may actually contribute to carcinogenesis by binding with TP53.

PATHOGENESIS. The evidence linking immunopathogenetic mechanism with hepatocellular damage is much stronger in HBV infection than with HAV infection.

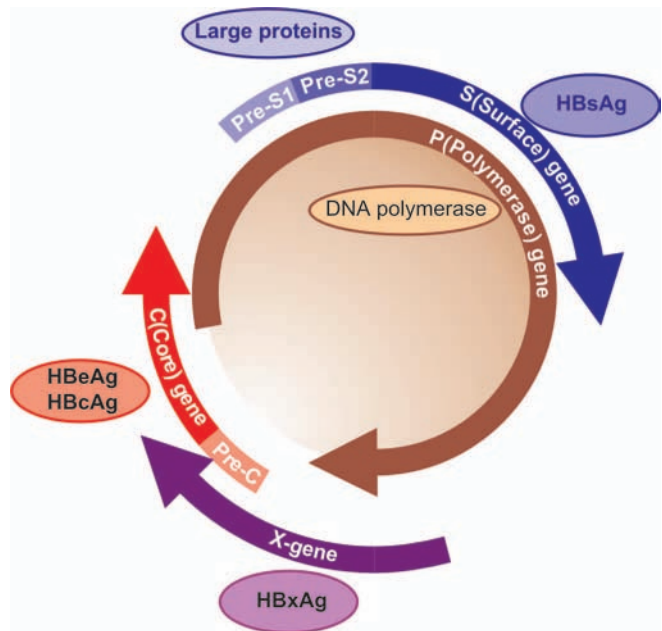


FIGURE 28.6

Genomic structure of Hepatitis B virus (Dane particle).

tion. In support of immune pathogenesis is the demonstration of several immunological markers (serologic as well as viral), and molecular and morphologic evidence that hepatocytic damage is initiated by virus-infected CD8+T cytotoxic cells.

Serologic and viral markers. Various immunological markers indicative of presence of HBV infection can be demonstrated in the sera as well as in the hepatocytes of infected individuals. These are as under (Fig. 28.7):

- 1. HBsAg.** In 1965, Blumberg and colleagues in Philadelphia found a lipoprotein complex in the serum of a multiple-transfused haemophiliac of Australian aborigine which was subsequently shown by them to be associated with serum hepatitis. This antigen was termed *Australia antigen* by them (In 1977, Blumberg was awarded the Nobel Prize for his discovery). The term Australia antigen is now used synonymous with hepatitis B surface antigen (HBsAg). HBsAg appears early in the blood after about 6 weeks of infection and its detection is an indicator of active HBV infection. It usually disappears in 3-6 months. Its persistence for more than 6 months implies a carrier state. HBsAg may also be demonstrated in the cell membrane of hepatocytes of carriers and chronic hepatitis patients by *Orcein staining* (orange positivity) but not in the hepatocytes during acute stage of illness.

- 2. Anti-HBs.** Specific antibody to HBsAg in serum called anti-HBs appears late, about 3 months after the onset. Anti-HBs response may be both IgM and IgG type. The prevalence rate of anti-HBs ranges from 10-15%. In these individuals it persists for life providing protection against reinfection with HBV.

- 3. HBeAg.** HBeAg derived from core protein is present transiently (3-6 weeks) during an acute attack. Its persistence beyond 10 weeks is indicative of development of chronic liver disease and carrier state.

- 4. Anti-HBe.** Antibody to HBeAg called anti-HBe appears after disappearance of HBeAg. Seroconversion from HBeAg to anti-HBe during acute stage of illness is a prognostic sign for resolution of infection.

- 5. HBcAg.** HBcAg derived from core protein cannot be detected in the blood. But HBcAg can be demonstrated in the nuclei of hepatocytes in carrier state and in chronic hepatitis patients by Orcein staining but not in the liver cells during acute stage.

- 6. Anti-HBc.** Antibody to HBcAg called anti-HBc can, however, be detected in the serum of acute hepatitis B patients during pre-icteric stage. Anti-HBc may be IgM or IgG class antibody. IgM anti-HBc persists for 4-6 months and is followed later by IgG anti-HBc. Thus detection of high titre of IgM anti-HBc is indicative of

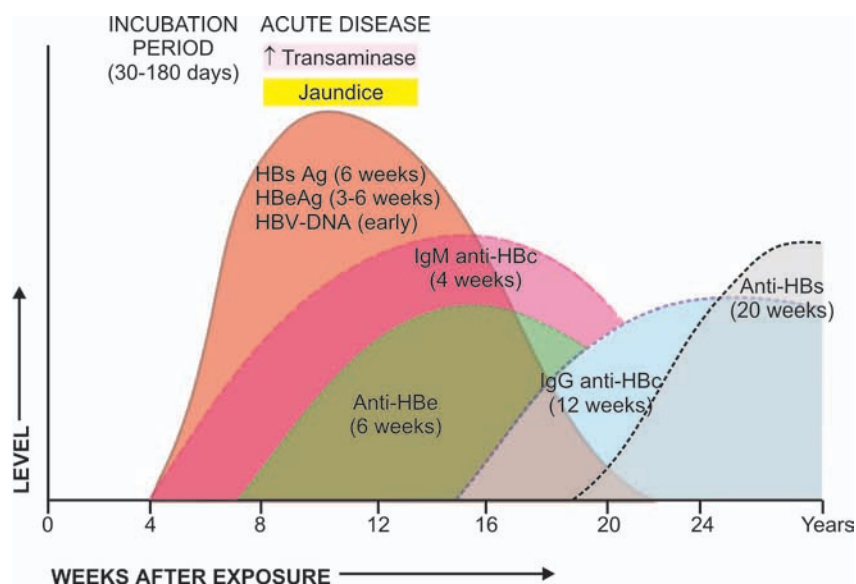


FIGURE 28.7

Sequence of serologic and viral markers in acute hepatitis B.

recent acute HBV infection, while elevated level of IgG anti-HBc suggests HBV infection in the remote past.

7. HBV-DNA. Detection of HBV-DNA by molecular hybridisation using the Southern blot technique is the most sensitive index of hepatitis B infection. It is present in pre-symptomatic phase and transiently during early acute stage.

Hepatitis D

Infection with delta virus (HDV) in the hepatocyte nuclei of HBsAg-positive patients is termed hepatitis D. HDV is a defective virus for which HBV is the helper. Thus, hepatitis D develops when there is concomitant hepatitis B infection. HDV infection and hepatitis B may be simultaneous (*co-infection*), or HDV may infect a chronic HBsAg carrier (*superinfection*) (Fig. 28.8):

■ With **coinfection**, acute hepatitis D may range from mild to fulminant hepatitis but fulminant hepatitis is more likely in such simultaneous delta infection. Chronicity rarely develops in coinfection.

■ With **superinfection**, (incubation period 30-35 days), chronic HBV infection gets worsened indicated by appearance of severe and fulminant acute attacks, progression of carrier stage to chronic delta hepatitis or acceleration towards cirrhosis. Hepatocellular carcinoma is, however, less common in HBsAg carriers with HDV infection.

HDV infection is worldwide in distribution though the incidence may vary in different countries. Endemic regions are Southern Europe, Middle-East, South India and parts of Africa. The high risk individuals for HDV infection are the same as for HBV infection i.e. intravenous drug abusers, homosexuals, transfusion recipients, and health care workers.

HEPATITIS DELTA VIRUS (HDV). The etiologic agent, HDV, is a small single-stranded RNA particle with a diameter of 36 nm. It is double-shelled—the outer shell consists of HBsAg and the inner shell consists of delta antigen provided by a circular RNA strand. It is highly infectious and can induce hepatitis in any HBsAg-positive host. HDV replication and proliferation takes place within the nuclei of liver cells. Markers for HDV infection include the following:

1. *HDV identification* in the blood and in the liver cell nuclei.
2. *HDAg* detectable in the blood and on fixed liver tissue specimens.
3. *Anti-HD antibody* in acute hepatitis which is initially IgM type and later replaced by IgG type anti-HD antibody which persists for life to confer immunity against reinfection.

PATHOGENESIS. HDV, unlike HBV, is thought to cause direct cytopathic effect on hepatocytes. However, there

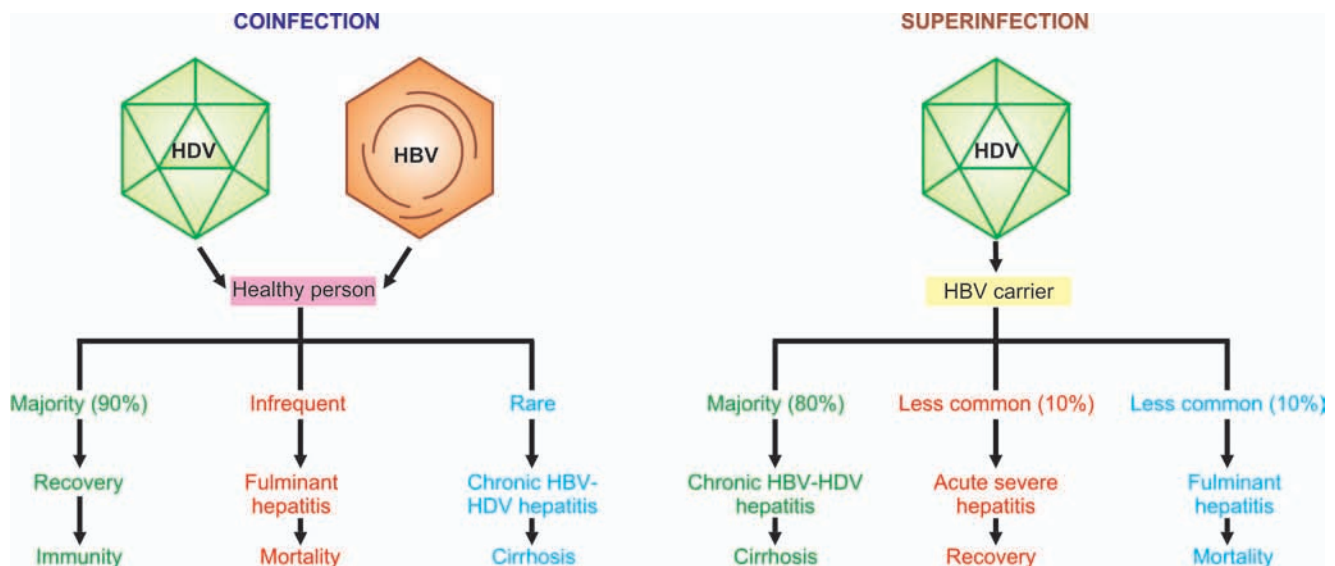


FIGURE 28.8

Consequences of coinfection versus superinfection in combined HDV-HBV infection.

are examples of transmission of HDV infection from individuals who themselves have not suffered from any attack of hepatitis, suggesting that it may not be always cytopathic. Thus, the exact mechanism remains unresolved.

Hepatitis C

The diagnosis of a third major category of hepatitis was earlier made after exclusion of infection with other known hepatitis viruses and was designated non-A, non-B (NANB) hepatitis. However, now this third type has been characterised and is called hepatitis C.

Hepatitis C infection is acquired by blood transfusions, blood products, haemodialysis, parenteral drug abuse and accidental cuts and needle-pricks in health workers. About 90% of post-transfusion hepatitis is of hepatitis C type. About 1-2% of volunteer blood donors and up to 5% of professional blood donors are carriers of HCV. Hepatitis C has an incubation period of 20-90 days (mean 50 days). Clinically, acute HCV hepatitis is milder than HBV hepatitis but HCV has a higher rate of progression to chronic hepatitis than HBV. Persistence of infection and chronic hepatitis are the key features of HCV. Occurrence of cirrhosis after 5 to 10 years and progression to hepatocellular carcinoma are other consequences of HCV infection. Currently, HCV is considered more important cause of chronic liver disease worldwide than HBV.

HEPATITIS C VIRUS (HCV). HCV is a single-stranded, enveloped RNA virus, having a diameter of 30-60 nm. HCV genome has about 3000 amino acids. The genomic organisation of HCV shows a 5' terminal end, C (capsid) region and the envelope regions E1 and E2 in the exons (Fig. 28.9).

The viral proteins result in corresponding serologic and virologic markers for HCV infection as under (Fig. 28.10):

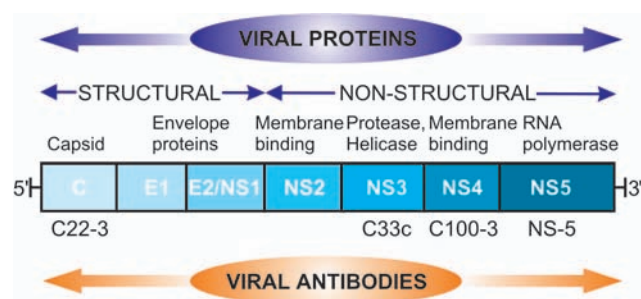


FIGURE 28.9

Diagrammatic structure of hepatitis C virus.

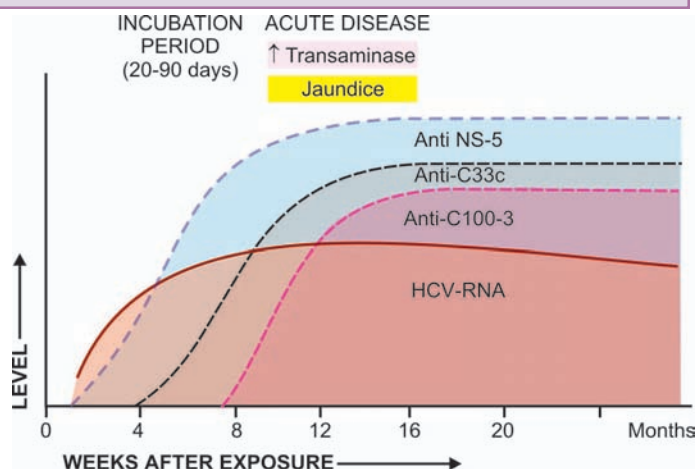


FIGURE 28.10

Sequence of serologic and viral markers of HCV infection.

1. *Anti-HCV antibodies.* Three generations of anti-HCV IgG assays are available:

- First generation antibodies are against C100-3 region proteins and appear 1 to 3 months after infection.
- Second generation antibodies are against C200 and C33c proteins and appear about one month earlier than the first generation.
- Third generation antibodies are against C22-3 and NS-5 region proteins and are detected even earlier.

2. *HCV-RNA.* HCV infection is, however, confirmed by HCV-RNA employing PCR technique which can be detected within a few days after exposure to HCV infection, much before appearance of anti-HCV and persists for the duration of HCV infection.

PATHOGENESIS. How HCV causes liver cell injury is not yet clearly established. HCV virions have not been identified in hepatocytes. Cell-mediated immune mechanism certainly play a role in hepatocytic injury due to HCV. Perhaps, HCV infection of lymphoid cells may play a role in immunologic injury to hepatocytes. In patients with chronic HCV hepatitis, HCV-specific CD4+ T cells and HLA-restricted CD8+ T cells have been identified. Viral and host crossreacting antibodies to liver and kidney microsomal antigen (anti-LKM) have been reported in a subset of patients that explains the association of autoimmune hepatitis and HCV hepatitis.

Hepatitis E

Hepatitis E is an enterically-transmitted virus, previously labelled as epidemic or enterically transmitted type of non-A non-B hepatitis. The infection occurs in young or middle-aged individuals, primarily in India, other Asian countries, Africa and central America.

The infection is generally acquired by contamination of water supplies such as after monsoon flooding. However, compared with HAV, secondary person-to-person infection does not occur with HEV. Thus HEV has some common epidemiologic features with HAV. HEV infection has a particularly high mortality in pregnant women but is otherwise a self-limited disease and has not been associated with chronic liver disease.

HEPATITIS E VIRUS (HEV). HEV is a single-stranded 32-34 nm, icosahedral non-enveloped virus. The virus has been isolated from stools, bile and liver of infected persons. Serologic markers for HEV include the following:

1. Anti-HEV antibodies of both IgM and IgG class.
2. HEV-RNA.

However, testing for these markers for HEV is currently not available.

Hepatitis G

A virus distinct from the foregoing hepatitis viruses has been designated separately by two groups of workers as hepatitis G (HGV) and hepatitis F (HFV) virus. Like HCV, HGV is a blood-borne infection and may cause acute and chronic viral hepatitis.

HEPATITIS G VIRUS (HGV). HGV is a single-stranded RNA virus. At present HGV infection has been found in blood donors and is transmitted by blood transfusion. The virus has been identified by PCR amplification technique.

CLINICOPATHOLOGIC SPECTRUM

Among of the various etiologic types of hepatitis, evidence linking HBV and HCV infection with the spectrum of clinicopathologic changes is stronger than with other hepatotropic viruses. The typical pathologic changes of hepatitis by all hepatotropic viruses are virtually similar. HAV and HEV, however, do not have a carrier stage or cause chronic hepatitis. The various clinical patterns and pathologic consequences of different hepatotropic viruses can be considered under the following headings:

- i) Carrier state
- ii) Asymptomatic infection
- iii) Acute hepatitis
- iv) Chronic hepatitis
- v) Fulminant hepatitis (Submassive to massive necrosis)

In addition, progression to cirrhosis and association with hepatocellular carcinoma are known to occur in certain types of hepatitis which are discussed separately later.

I. Carrier State

An asymptomatic individual without manifest disease, harbouring infection with hepatotropic virus and capable of transmitting it is called carrier state. There can be 2 types of carriers:

1. An '*asymptomatic healthy carrier*' who does not suffer from ill-effects of the virus infection.
2. An '*asymptomatic carrier with chronic disease*' capable of transmitting the organisms.

As stated before, hepatitis A and E do not produce the carrier state. Hepatitis B is responsible for the largest number of carriers in the world, while concomitant infection with HDV more often causes progressive disease rather than an asymptomatic carrier state. An estimated 2-3% of the general population are asymptomatic carriers of HCV. Data on HBV carrier state reveal role of 2 important factors rendering the individual more vulnerable to harbour the organisms—*early age at infection* and *impaired immunity*. Whereas approximately 10% of adults contracting hepatitis B infection develop carrier state, 90% of infected neonates fail to clear HBsAg from the serum within 6 months and become HBV carriers.

Clinical recognition of carrier state of HBV is more frequently done by detection of HBsAg in the serum and less often by other markers such as HBeAg, HBcAg and antibodies. Concomitant infection of HDV with HBV depends upon the demonstration of anti-HD.

PATHOLOGIC CHANGES. Carriers of HBV may or may not show changes on liver biopsy.

■ *Healthy HBV carriers* may show no changes or minor hepatic change such as presence of finely granular, ground-glass, eosinophilic cytoplasm as evidence of HBsAg.

■ *Asymptomatic carriers with chronic disease* may show changes of chronic hepatitis and even cirrhosis.

II. Asymptomatic Infection

These are cases who are detected incidentally to have infection with one of the hepatitis viruses as revealed by their raised serum transaminases or by detection of the presence of antibodies but are otherwise asymptomatic.

III. Acute Hepatitis

The most common consequence of all hepatotropic viruses is acute inflammatory involvement of the entire liver. In general, type A, B, C, D and E run similar clinical course and show identical pathologic findings.

Clinically, acute hepatitis is categorised into 4 phases: incubation period, pre-icteric phase, icteric phase and post-icteric phase.

1. Incubation period: It varies among different hepatotropic viruses: for hepatitis A it is about 4 weeks (15-45 days); for hepatitis B the average is 10 weeks (30-180 days); for hepatitis D about 6 weeks (30-50 days); for hepatitis C the mean incubation period is about 7 weeks (20-90 days), and for hepatitis E it is 2-8 weeks (15-60 days). The patient remains asymptomatic during incubation period but the infectivity is highest during the last days of incubation period.

2. Pre-icteric phase: This phase is marked by prodromal constitutional symptoms that include anorexia, nausea, vomiting, fatigue, malaise, distaste for smoking, arthralgia and headache. There may be low-grade fever preceding the onset of jaundice, especially in hepatitis A. The earliest laboratory evidence of hepatocellular injury in pre-icteric phase is the elevation of transaminases.

3. Icteric phase: The prodromal period is heralded by the onset of clinical jaundice and the constitutional symptoms diminish. Other features include dark-coloured urine due to bilirubinuria, clay-coloured stools due to cholestasis, pruritus as a result of elevated serum bile acids, loss of weight and abdominal discomfort due to enlarged, tender liver. The diagnosis is based on deranged liver function tests (e.g. elevated levels of serum bilirubin, transaminases and alkaline phosphatase; prolonged prothrombin time and hyperglobulinaemia) and serologic detection of hepatitis antigens and antibodies.

4. Post-icteric phase: The icteric phase lasting for about 1 to 4 weeks is usually followed by clinical and biochemical recovery in 2 to 12 weeks. The recovery phase is more prolonged in hepatitis B and hepatitis C. Up to 1% cases of acute hepatitis may develop severe form of the disease (fulminant hepatitis); and 5-10% of cases progress on to chronic hepatitis. Evolution into the carrier state (except in HAV and HEV infection) has already been described above.

PATHOLOGIC CHANGES. *Grossly*, the liver is slightly enlarged, soft and greenish.

Histologically, the changes are as follows (Fig. 28.11):

1. Hepatocellular injury: There may be variation in the degree of liver cell injury but it is most marked in zone 3 (centrilobular zone):

- i) Mildly injured hepatocytes appear swollen with granular cytoplasm which tends to condense around the nucleus (*ballooning degeneration*).
- ii) Others show acidophilic degeneration in which the cytoplasm becomes intensely eosinophilic, the nucleus becomes small and pyknotic and is eventually extruded from the cell, leaving behind necrotic, acidophilic mass called *Councilman body* or *acidophil body* by the process known as apoptosis.
- iii) Another type of hepatocellular necrosis is *dropout necrosis* in which isolated or small clusters of hepatocytes undergo lysis.
- iv) *Bridging necrosis* is a more severe form of hepatocellular injury in acute viral hepatitis and may progress to fulminant hepatitis or chronic hepatitis (page 369). Bridging necrosis is characterised by bands of necrosis linking portal tracts to central hepatic veins, one central hepatic vein to another, or a portal tract to another tract.

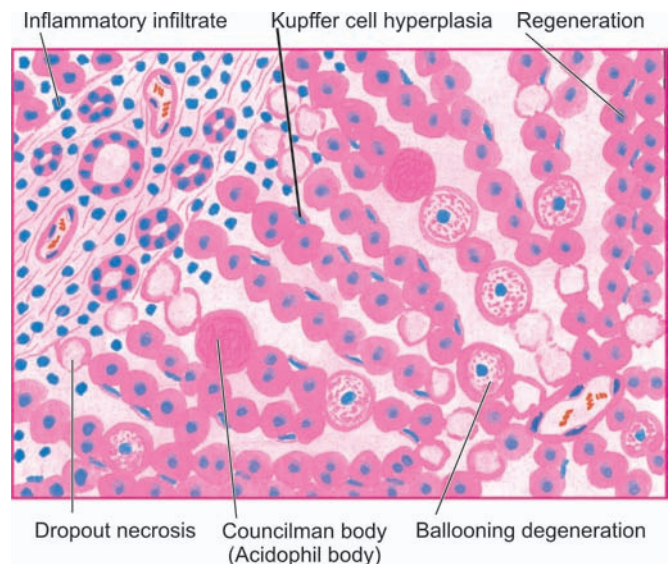


FIGURE 28.11

Acute viral hepatitis. The predominant histologic changes are: variable degree of necrosis of hepatocytes, most marked in zone 3 (centrilobular); and mononuclear cellular infiltrate, chiefly in zone 1 (portal area). Mild degree of liver cell necrosis is seen as ballooning degeneration while acidophilic Councilman bodies are indicative of more severe liver cell injury.

- 2. Inflammatory infiltrate:** There is infiltration by mononuclear inflammatory cells, usually in the portal tracts, but may permeate into the lobules.
- 3. Kupffer cell hyperplasia:** There is reactive hyperplasia of Kupffer cells many of which contain phagocytosed cellular debris, bile pigment and lipofuscin granules.
- 4. Cholestasis:** Biliary stasis is usually not severe in viral hepatitis and may be present as intracytoplasmic bile pigment granules.
- 5. Regeneration:** As a result of necrosis of hepatocytes, there is lobular disarray. Surviving adjacent hepatocytes undergo regeneration and hyperplasia. If the necrosis causes collapse of reticulin framework of the lobule, healing by fibrosis follows, distorting the lobular architecture.

The above histologic changes apply generally to viral hepatitis by various types of hepatotropic viruses and by HBV in particular. In general, it is not possible to distinguish histologically between viral hepatitis of various etiologies, the following morphologic features may help in giving an etiologic clue:

- **HAV hepatitis** is a panlobular involvement by heavy inflammatory infiltrate compared to other types.
- **HCV hepatitis** causes milder necrosis, with fatty change in hepatocytes, presence of lymphoid aggregates in the portal triads and degeneration of bile duct epithelium.

N. Chronic Hepatitis

Chronic hepatitis is defined as continuing or relapsing hepatic disease for more than 6 months with symptoms alongwith biochemical, serologic and histopathologic evidence of inflammation and necrosis. Majority of cases of chronic hepatitis are the result of infection with hepatotropic viruses—hepatitis B, hepatitis C and combined hepatitis B and hepatitis D infection. However, some non-viral causes of chronic hepatitis include: Wilson's disease, α -1-antitrypsin deficiency, chronic alcoholism, drug-induced injury and autoimmune diseases. The last named gives rise to *autoimmune or lupoid hepatitis* which is characterised by positive serum autoantibodies (e.g. antinuclear, anti-smooth muscle and anti-mitochondrial) and a positive LE cell test but negative for serologic markers of viral hepatitis.

Until recent years, prediction of prognosis of chronic hepatitis used to be made on the basis of morphology which divided it into 2 main types—*chronic persistent* and *chronic active (aggressive) hepatitis*. A third form,

chronic lobular hepatitis is distinguished separately by some as mild form of lobular inflammation without inflammation of portal tracts but these cases often recover completely. However, subsequent studies have revealed that morphologic subtypes do not necessarily correlate with the prognosis since the disease is not essentially static but may vary from mild form to severe and *vice versa*. Besides, two other factors which determine the vulnerability of a patient of viral hepatitis to develop chronic hepatitis are: *impaired immunity* and *extremes of age* at which the infection is first contracted.

Currently, chronic hepatitis is classified on the basis of etiology. The frequency and severity with which hepatotropic viruses cause chronic hepatitis varies with the organisms as under:

- **HCV infection** accounts for 40-60% cases of chronicity in adults. HCV infection is particularly associated with progressive form of chronic hepatitis that may evolve into cirrhosis.
- **HBV** causes chronic hepatitis in 90% of infected infants and in about 5% adult cases of hepatitis B.
- **HDV superinfection** on HBV carrier state may be responsible for chronic hepatitis in 10-40% cases.
- **HAV and HEV** do not produce chronic hepatitis.

PATHOLOGIC CHANGES. The pathologic features are common to both HBV and HCV infection and include the following lesions (Fig. 28.12).

1. Piecemeal necrosis. Piecemeal necrosis is defined as periportal destruction of hepatocytes at the limiting plate (*piecemeal* = piece by piece). Its features in chronic hepatitis are:

- i) Necrosed hepatocytes at the limiting plate in periportal zone.
- ii) Interface hepatitis due to expanded portal tract by infiltration of lymphocytes, plasma cells and macrophages.
- iii) Expanded portal tracts are often associated with proliferating bile ductules as a response to liver cell injury.

2. Portal tract lesions. All forms of chronic hepatitis are characterised by variable degree of changes in the portal tract.

- i) Inflammatory cell infiltration by lymphocytes, plasma cells and macrophages (triaditis).
- ii) Proliferated bile ductules in the expanded portal tracts.
- iii) Additionally, chronic hepatitis C may show lymphoid aggregates or follicles with reactive germinal centre and infiltration of inflammatory cells in the damaged bile duct epithelial cells.

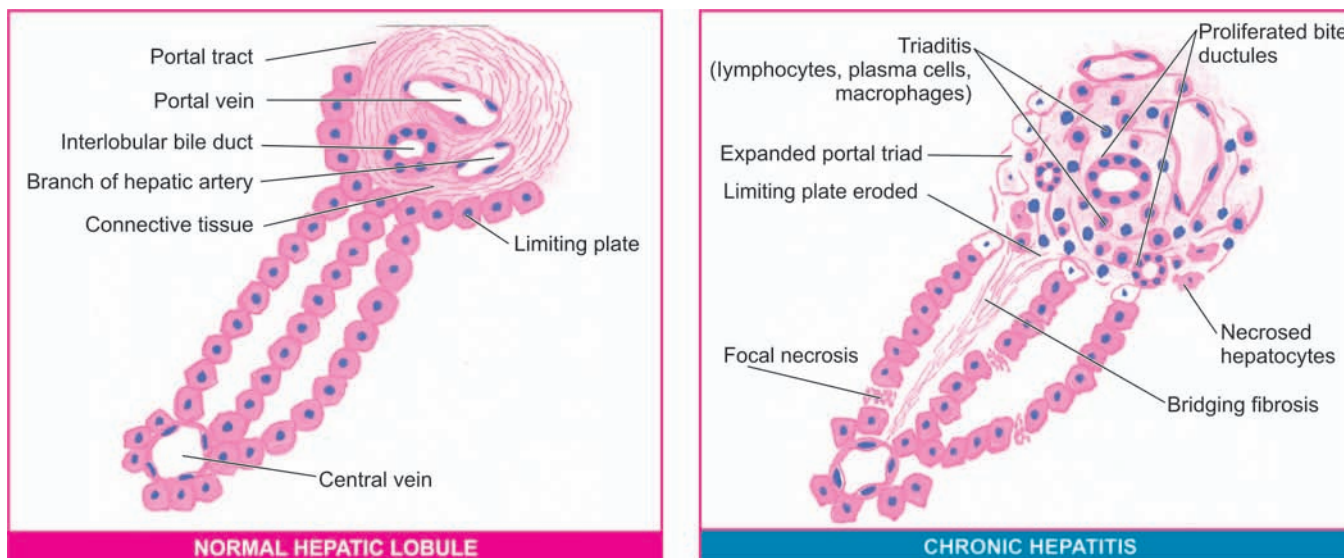


FIGURE 28.12

Diagrammatic representation of pathologic changes in chronic hepatitis.

3. Intralobular lesions. Generally, the architecture of lobule is retained in mild to moderate chronic hepatitis.

- i) There are focal areas of necrosis and inflammation within the hepatic parenchyma.
- ii) Scattered acidophilic bodies in the lobule.
- iii) Kupffer cell hyperplasia.
- iv) More severe form of injury shows bridging necrosis (i.e. bands of necrosed hepatocytes that may bridge portal tract-to-central vein, central vein-to-central vein, and portal tract-to-portal tract).
- v) Regenerative changes in hepatocytes in cases of persistent hepatocellular necrosis.
- vi) Cases of chronic hepatitis C show moderate fatty change.
- vii) Cases of chronic hepatitis B show scattered ground-glass hepatocytes indicative of abundance of HBsAg in the cytoplasm.

4. Bridging fibrosis. The onset of fibrosis in chronic hepatitis from the area of interface hepatitis and bridging necrosis is a feature of irreversible damage.

- i) At first, there is periportal fibrosis at the sites of interface hepatitis giving the portal tract stellate-shaped appearance.
- ii) Progressive cases show bridging fibrosis connecting portal tract-to-portal tract or portal tract-to-central vein traversing the lobule.
- iii) End stage of chronic hepatitis is characterised by dense collagenous septa destroying lobular architec-

ture and forming nodules resulting in postnecrotic cirrhosis.

As prognostic indicator of chronic hepatitis, criteria have been evolved to classify chronic hepatitis by giving **activity score** (maximum score of 22; ranging from minimal/mild to moderate and severe) based on the following features:

- periportal necrosis i.e. piecemeal necrosis and/or bridging necrosis;
- extent and depth of inflammation; and
- extent and density of fibrosis.

CLINICAL FEATURES. The clinical features of chronic hepatitis are quite variable ranging from mild disease to full blown picture of cirrhosis.

- i) Mild chronic hepatitis shows only slight but persistent elevation of transaminases ('transaminitis') with fatigue, malaise and loss of appetite.
- ii) Other cases may show mild hepatomegaly, hepatic tenderness and mild splenomegaly.
- iii) Laboratory findings may reveal prolonged prothrombin time, hyperbilirubinaemia, hyperglobulinaemia and markedly elevated alkaline phosphatase.
- iv) Systemic features of circulating immune complexes due to HBV and HCV infection may produce features of immune complex vasculitis, glomerulonephritis and cryoglobulinaemia in a proportion of cases.

However, clinical features do not correlate with morphologic appearance of the liver biopsy. Some

patients may have mild form of disease without progressing for several years while others may show rapid evolution into cirrhosis with its complications over a period of few years. Patients of long-standing HBV and HCV infection are known to evolve into hepatocellular carcinoma.

V. Fulminant Hepatitis (Submassive to Massive Necrosis)

Fulminant hepatitis is the most severe form of acute hepatitis in which there is rapidly progressive hepatocellular failure. Two patterns are recognised—*submassive necrosis* having a less rapid course extending up to 3 months; and *massive necrosis* in which the liver failure is rapid and fulminant occurring in 2-3 weeks.

Fulminant hepatitis of either of the two varieties can occur from viral and non-viral etiologies:

■ *Acute viral hepatitis* accounts for about half the cases, most often from HBV and HCV; less frequently from combined HBV-HDV and rarely from HAV. However, HEV infection is a serious complication in pregnant women. In addition, herpesvirus can also cause serious viral hepatitis.

■ *Non-viral causes* include acute hepatitis due to drug toxicity (e.g. acetaminophen, non-steroidal anti-inflammatory drugs, isoniazid, halothane and antidepressants), poisonings, hypoxic injury and massive infiltration of malignant tumours into the liver.

The patients present with features of hepatic failure with hepatic encephalopathy. The mortality rate is high if hepatic transplantation is not undertaken.

PATHOLOGIC CHANGES. *Grossly*, the liver is small and shrunken, often weighing 500-700 gm. The capsule is loose and wrinkled. The sectioned surface shows diffuse or random involvement of hepatic lobes. There are extensive areas of muddy-red and yellow necrosis (previously called *acute yellow atrophy*) and patches of green bile staining.

Microscopically, two forms of fulminant necrosis are distinguished—submassive and massive necrosis.

i) In **submassive necrosis**, large groups of hepatocytes in zone 3 (centrilobular area) and zone 2 (mid zone) are wiped out leading to a collapsed reticulin framework. Regeneration in submassive necrosis is more orderly and may result in restoration of normal architecture.

ii) In **massive necrosis**, the entire liver lobules are necrotic. As a result of loss of hepatic parenchyma, all that is left is the collapsed and condensed reticulin framework and portal tracts with proliferated bile

ductules plugged with bile. Inflammatory infiltrate is scanty. Regeneration, if it takes place, is disorderly forming irregular masses of hepatocytes. Fibrosis is generally not a feature of fulminant hepatitis (Fig. 28.13).

The clinicopathologic course in two major forms of hepatitis, HBV and HCV, is summarised in Fig. 28.14.

CIRRHOSIS

Cirrhosis of the liver is a diffuse disease having the following 4 features:

1. It involves the entire liver.
2. The normal lobular architecture of hepatic parenchyma is disorganised.
3. There is formation of nodules separated from one another by irregular bands of fibrosis.
4. It occurs following hepatocellular necrosis of varying etiology so that there are alternate areas of necrosis and regenerative nodules.

However, regenerative nodules are not essential for diagnosis of cirrhosis since biliary cirrhosis and haemochromatosis have little regeneration. The fibrosis once developed is irreversible.

In the Western world, cirrhosis of the liver is one of the ten leading causes of death.

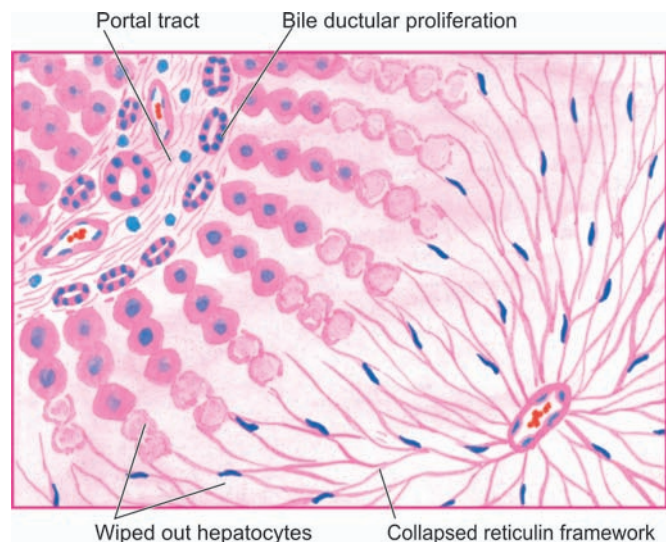


FIGURE 28.13

Fulminant hepatitis. There is complete wiping out of liver lobules with only collapsed reticulin framework left out in their place. There is no significant inflammation or fibrosis.

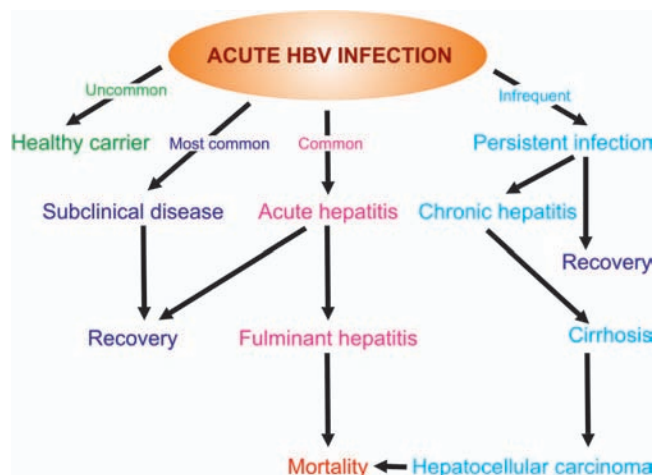


FIGURE 28.14

Clinicopathologic course of HBV and HCV infection.

PATHOGENESIS

Irrespective of the etiology, cirrhosis in general is initiated by hepatocellular necrosis. Continued destruction of hepatocytes causes collapse of normal lobular hepatic parenchyma followed by *fibrosis* around necrotic liver cells and proliferated ductules and there is formation of compensatory *regenerative nodules*.

FIBROGENESIS. The mechanism of fibrosis that may be portal-central, portal-portal, or both, is by increased synthesis of all types of collagen and increase in the number of collagen-producing cells. Development of fibrosis leads to proliferation of fat-storing Ito cells underlying the sinusoidal epithelium which become transformed into myofibroblasts and fibrocytes. Besides collagen, two glycoproteins, fibronectin and laminin, are deposited in excessive amounts in area of liver cell damage. The nature of factors acting as stimulants for fibrosis is not clearly known, but possible candidate mediators are lymphokines and monokines.

REGENERATIVE NODULE. The cause of compensatory proliferation of hepatocytes to form regenerative nodules is obscure. Possibly, growth factors, chaperones and hormonal imbalance, play a role in regeneration.

CLASSIFICATION

Cirrhosis can be classified on the basis of morphology and etiology (Table 28.5).

A. MORPHOLOGIC CLASSIFICATION. There are 3 morphologic types of cirrhosis—micronodular, macro-

TABLE 28.5: Classification of Cirrhosis.

A. MORPHOLOGIC	B. ETIOLOGIC
I. Micronodular (nodules less than 3 mm)	1. Alcoholic cirrhosis (the most common, 60-70%)
II. Macronodular (nodules more than 3 mm)	2. Post-necrotic cirrhosis (10%)
III. Mixed	3. Biliary cirrhosis (5-10%)
	4. Pigment cirrhosis in haemochromatosis (5%)
	5. Cirrhosis in Wilson's disease
	6. Cirrhosis in α -1-antitrypsin deficiency
	7. Cardiac cirrhosis
	8. Indian childhood cirrhosis (ICC)
	9. Miscellaneous forms of cirrhosis
	10. Cryptogenic cirrhosis

nodular and mixed. Each of these forms may have an active and inactive form.

■ An *active form* is characterised by continuing hepatocellular necrosis and inflammatory reaction, a process that closely resembles chronic hepatitis.

■ An *inactive form*, on the other hand, has no evidence of continuing hepatocellular necrosis and has sharply-defined nodules of surviving hepatic parenchyma without any significant inflammation.

1. Micronodular cirrhosis. In micronodular cirrhosis, the nodules are usually regular and small, *less than 3 mm* in diameter. There is diffuse involvement of all the hepatic lobules forming nodules by thick fibrous septa which may be portal-portal, portal-central, or both. The micronodular cirrhosis includes etiologic types of alcoholic cirrhosis, nutritional cirrhosis and Laennec's cirrhosis and represents impaired capacity for regrowth as seen in alcoholism, malnutrition, severe anaemia and old age.

2. Macronodular cirrhosis. In this type, the nodules are of variable size and are generally *larger than 3 mm* in diameter. The pattern of involvement is more irregular than in micronodular cirrhosis, sparing some portal tracts and central veins, and more marked evidence of regeneration. Macronodular cirrhosis corresponds to post-necrotic or post-hepatic cirrhosis of the etiologic classification.

3. Mixed cirrhosis. In mixed type, some parts of the liver show micronodular appearance while other parts show macronodular pattern. All the portal tracts and central veins are not involved by fibrosis but instead some of them are spared. Mixed pattern is a kind of incomplete expression of micronodular cirrhosis.



GLOMERULONEPHRITIS

Glomerular diseases encompass a large and clinically significant group of renal diseases. Glomerulonephritis (GN) or Bright's disease is the term used for diseases that primarily involve the renal glomeruli. It is convenient to classify glomerular diseases into 2 broad groups:

I. *Primary glomerulonephritis* in which the glomeruli are the predominant site of involvement.

II. *Secondary glomerular diseases* include certain systemic and hereditary diseases which secondarily affect the glomeruli.

Though this division is widely followed, it is somewhat arbitrary since many primary forms of glomerulonephritis have systemic effects, and many systemic diseases may initially present with glomerular involvement. Many classifications of different types of glomerulonephritis have been described, but most widely accepted classification is based on clinical presentation and pathologic changes in the glomeruli given in Table 29.1.

CLINICAL MANIFESTATIONS

The clinical presentation of glomerular disease is quite variable but in general four features—proteinuria, haematuria, hypertension and disturbed excretory function, are present in varying combinations depending upon the underlying condition. A firm diagnosis, however, can be established by examination of renal biopsy under light, electron and immunofluorescence microscopy.

A number of clinical syndromes are recognised in glomerular diseases. The following are six major glomerular syndromes commonly found in different glomerular diseases:

- nephritic and nephrotic syndromes;
- acute and chronic renal failure;
- asymptomatic proteinuria and haematuria.

These are briefly described below.

I. ACUTE NEPHRITIC SYNDROME. This is the acute onset of haematuria, proteinuria, hypertension, oedema and oliguria following an infective illness about 10 to 20 days earlier.

TABLE 29.1: Clinicopathologic Classification of Glomerular Diseases.

I. Primary Glomerulonephritis
1. Acute GN
i) Post-streptococcal
ii) Non-streptococcal
2. Rapidly progressive GN
3. Minimal change disease
4. Membranous GN
5. Membrano-proliferative GN
6. Focal proliferative GN
7. Focal segmental glomerulosclerosis (FSGS)
8. IgA nephropathy
9. Chronic glomerulonephritis
II. Secondary Systemic Glomerular Diseases
1. Lupus nephritis (SLE)
2. Diabetic nephropathy
3. Amyloidosis
4. Polyarteritis nodosa
5. Wegener's granulomatosis
6. Goodpasture's syndrome
7. Henoch-Schönlein purpura
8. Systemic infectious diseases (<i>bacterial</i> e.g. bacterial endocarditis, syphilis, leprosy; <i>viral</i> e.g. HBV, HCV, HIV; <i>parasitic</i> e.g. falciparum malaria, filariasis)
9. Idiopathic mixed cryoglobulinaemia
III. Hereditary Nephritis
1. Alport's syndrome
2. Fabry's disease
3. Nail-patella syndrome

1. The **haematuria** is generally slight giving the urine smoky appearance and erythrocytes are detectable by microscopy or by chemical testing for haemoglobin. Appearance of red cell casts is another classical feature of acute nephritic syndrome.

2. The **proteinuria** is mild (less than 3 gm per 24 hrs) and is usually non-selective (*nephritic range proteinuria*).

3. **Hypertension** is variable depending upon the severity of the glomerular disease but is generally mild.

4. **Oedema** in nephritic syndrome is usually mild and results from sodium and water retention (page 191).

5. **Oliguria** is variable and reflects the severity of glomerular involvement.

TABLE 29.2: Causes of Acute Nephritic Syndrome.

- | |
|--------------------------------------|
| I. Primary Glomerulonephritis |
| 1. Acute GN |
| i) Post-streptococcal |
| ii) Non-streptococcal |
| 2. Rapidly progressive GN |
| 3. Membranoproliferative GN |
| 4. Focal GN |
| 5. IgA nephropathy |
| II. Systemic Diseases |
| 1. SLE |
| 2. Polyarteritis nodosa |
| 3. Wegener's granulomatosis |
| 4. Henoch-Schönlein purpura |
| 5. Cryoglobulinaemia |

The underlying causes of acute nephritic syndrome may be primary glomerulonephritic diseases (classically acute glomerulonephritis and rapidly progressive glomerulonephritis) or certain systemic diseases (Table 29.2).

II. NEPHROTIC SYNDROME Nephrotic syndrome is a group of diseases having different pathogenesis and characterised by clinical findings of massive proteinuria, hypoalbuminaemia, oedema, hyperlipidaemia, lipiduria, and hypercoagulability.

1. Heavy proteinuria (protein loss of more than 3 gm per 24 hrs) is the chief characteristic of nephrotic syndrome (*nephrotic range proteinuria*). In children, protein loss is correspondingly less. A small amount of protein (20 to 150 mg/day) normally passes through the glomerular filtration barrier and is reabsorbed by the tubules. But in case of increased glomerular permeability to plasma proteins, excess of protein is filtered out exceeding the capacity of tubules for reabsorption and, therefore, appears in the urine. Another feature of protein loss is its 'selectivity'. A *highly-selective proteinuria* consists mostly of loss of low molecular weight proteins, while a *poorly-selective proteinuria* is loss of high molecular weight proteins in the urine. In nephrotic syndrome, proteinuria mostly consists of loss of albumin (molecular weight 66,000) in the urine.

2. Hypoalbuminaemia is produced primarily consequent to urinary loss of albumin, and partly due to increased renal catabolism and inadequate hepatic synthesis of albumin. Often, the plasma albumin level is 1 to 3 gm/dl (normal 3.5 to 5.5 gm/dl) and there is reversed albumin-globulin ratio. The concentration of other proteins in the plasma such as immunoglobulins, clotting factors and antithrombin may fall rendering these patients more vulnerable to infections and thrombotic and thromboembolic complications.

3. Oedema in nephrotic syndrome appears due to fall in colloid osmotic pressure consequent upon hypoalbuminaemia. Sodium and water retention further contribute to oedema. Nephrotic oedema is usually peripheral but in children facial oedema may be more prominent (page 190).

4. Hyperlipidaemia is a frequent accompaniment of nephrotic syndrome. The exact mechanism of its genesis is not clear. It is hypothesised that the liver faced with the stress of massive protein synthesis in response to heavy urinary protein loss, also causes increased synthesis of lipoproteins. There are increased blood levels of total lipids, cholesterol, triglycerides, VLDL and LDL but decrease in HDL. Low blood level of HDL is partly due to its loss in the urine.

5. Lipiduria occurs following hyperlipidaemia due to excessive leakiness of glomerular filtration barrier.

6. Hypercoagulability. Patients with nephrotic syndrome may develop spontaneous arterial or venous thrombosis, renal vein thrombosis and pulmonary embolism as a result of various factors. These include: increased urinary loss of antithrombin III, hyperfibrinogenemia due to increased synthesis in the liver, decreased fibrinolysis, increased platelet aggregation and altered levels of protein C and S.

The causes of nephrotic syndrome are diverse and are listed in Table 29.3. The morphology of individual types is described later. But it must be mentioned here that:

■ in *children*, primary glomerulonephritis is the cause in majority of cases of the nephrotic syndrome, most frequently *lipoid nephrosis* (65%); and

■ in *adults*, on the other hand, systemic diseases (diabetes, amyloidosis and SLE) are more frequent causes of nephrotic syndrome. The most common primary glomerular disease in adults is *membranous glomerulonephritis* (40%).

III. ACUTE RENAL FAILURE. As already described above, acute renal failure (ARF) is characterised by rapid decline in renal function. ARF has many causes including glomerular disease, principally rapidly progressive GN and acute diffuse proliferative GN.

IV. CHRONIC RENAL FAILURE. Glomerular causes of chronic renal failure (CRF) have already been described above. These cases have advanced renal impairment progressing over years and is detected by significant proteinuria, haematuria, hypertension and azotaemia. Such patients generally have small contracted kidneys as a result of chronic glomerulonephritis.

TABLE 29.3: Causes of Nephrotic Syndrome.

I. Primary Glomerulonephritis
1. Minimal change disease (<i>most common in children</i>)
2. Membranous GN (<i>most common in adults</i>)
3. Membranoproliferative GN
4. Focal segmental glomerulosclerosis
5. Focal GN
6. IgA nephropathy
II. Systemic Diseases
1. Diabetes mellitus
2. Amyloidosis
3. SLE
III. Systemic Infections
1. Viral infections (HBV, HCV, HIV)
2. Bacterial infections (bacterial endocarditis, syphilis, leprosy)
3. Protozoa and parasites (<i>P. falciparum</i> malaria, filariasis)
IV. Hypersensitivity Reactions
1. Drugs (heavy metal compounds like gold and mercury, other drugs like penicillamine, trimethadione and tolbutamide, heroin addiction)
2. Bee stings, snake bite, poison ivy
V. Malignancy
1. Carcinomas
2. Myeloma
3. Hodgkin's disease
VI. Pregnancy
Toxaemia of pregnancy
VII. Circulatory Disturbances
1. Renal vein thrombosis
2. Constrictive pericarditis
VIII. Hereditary Diseases
1. Alport's disease
2. Fabry's disease
3. Nail-patella syndrome

V. ASYMPTOMATIC PROTEINURIA. Presence of proteinuria unexpectedly in a patient may be unrelated to renal disease (e.g. exercise-induced, extreme lordosis

and orthostatic proteinuria), or may indicate an underlying mild glomerulonephritis. Association of asymptomatic haematuria, hypertension or impaired renal function with asymptomatic proteinuria should raise strong suspicion of underlying glomerulonephritis.

VI. ASYMPTOMATIC HAEMATURIA. Asymptomatic microscopic haematuria is common in children and young adolescents and has many diverse causes such as diseases of the glomerulus, renal interstitium, calyceal system, ureter, bladder, prostate, urethra, and underlying bleeding disorder, congenital abnormalities of the kidneys or neoplasia. Glomerular haematuria is indicated by the presence of red blood cells, red cell casts and haemoglobin in the urine. Glomerular haematuria is frequently associated with asymptomatic proteinuria.

PATHOGENESIS OF GLOMERULAR INJURY

Most forms of primary GN and many of the secondary glomerular diseases in human beings have immunologic pathogenesis. This view is largely based on immunofluorescence studies of GN in humans which have revealed glomerular deposits of immunoglobulins and complement in patterns that closely resemble those of experimental models. Non-immunologic mechanisms, however, play some role in certain forms of glomerular damage as discussed later.

The consequences of injury at different sites within the glomerulus in various glomerular diseases can be assessed when compared with the normal physiologic role of the main cells involved i.e. *endothelial*, *mesangial*, *visceral epithelial*, and *parietal epithelial cells* as well as of the GBM (Table 29.4).

Immunologic mechanisms underlying glomerular injury are primarily *antibody-mediated* (immune-complex disease). There is evidence to suggest that *cell-mediated immune reactions* in the form of delayed type hypersensitivity can also cause glomerular injury. In addition, a few secondary mechanisms and some non-immuno-

TABLE 29.4: Relationship of Physiologic Role of Glomerular Components with Consequences in Glomerular Injury.

COMPONENT	PHYSIOLOGIC FUNCTION	CONSEQUENCE OF INJURY	RELATED GLOMERULAR DISEASE
1. <i>Endothelial cells</i>	i) Maintain glomerular perfusion ii) Prevent leucocyte adhesion iii) Prevent platelet aggregation	Vasoconstriction Leucocyte infiltration Intravascular microthrombi	Acute renal failure Focal/diffuse proliferative GN Thrombotic microangiopathies
2. <i>Mesangial cells</i>	Control glomerular filtration	Proliferation and increased matrix	Membranoproliferative GN
3. <i>Visceral epithelial cells</i>	Prevent plasma protein filtration	Proteinuria	Minimal change disease, FSGS
4. <i>GBM</i>	Prevents plasma protein filtration	Proteinuria	Membranous GN
5. <i>Parietal epithelial cells</i>	Maintain Bowman's space	Crescent formation	RPGN

logic mechanisms are involved in the pathogenesis of some forms of glomerular diseases in human beings (Table 29.5).

I. IMMUNOLOGIC MECHANISMS

Experimental studies and observations in man have revealed that immunologic mechanisms, most importantly antigen-antibody complexes, underlie most forms of glomerular injury. The general principles of these mechanisms in different forms of glomerular diseases are discussed below, while more specific features are described under the specific types of GN.

A. Antibody-Mediated Glomerular Injury

1. IMMUNE COMPLEX DISEASE. Majority of cases of glomerular disease result from deposits of immune complexes (antigen-antibody complexes). The immune complexes are represented by *irregular or granular glomerular deposits* of immunoglobulins (IgG, IgM and IgA) and complement (mainly C3). Based on the experimental models and studies in human beings, the following 3 patterns of glomerular deposits of immune complexes in various glomerular diseases have been observed (Fig. 29.1):

i) *Exclusive mesangial deposits* are characterised by very mild form of glomerular disease.

ii) *Extensive subendothelial deposits* along the GBM are accompanied by severe hypercellular sclerosing glomerular lesions.

iii) *Subepithelial deposits* are seen between the outer surface of the GBM and the podocytes.

Deposits may be located at one or more of the above sites in any case of glomerular injury.

It was widely believed earlier that glomerular deposits result from circulating immune complexes. Now, it has been shown that glomerular deposits are formed by one of the following two mechanisms:

i) Local immune complex deposits. Formation of glomerular deposits of immune complex *in situ* occurs as a result of combination of antibodies with autologous non-basement membrane antigens or nonglomerular antigens planted on glomeruli. Currently, this mechanism is considered responsible for most cases of immune complex GN. Classic experimental model of *in situ* immune complex GN is *Heymann nephritis* (autologous immune complex nephritis) induced in rats by immunising animals with homologous preparations of proximal tubular brush border. The rats develop antibodies to brush border antigens and thereby membranous GN that closely resembles human membranous GN. The examples of *planted non-glomerular antigens* are cationic proteins, lectins, DNA, bacterial products (e.g.

TABLE 29.5: Pathogenetic Mechanisms in Glomerular Diseases.

MECHANISM	RELATED GLOMERULAR DISEASE
I. IMMUNOLOGIC MECHANISMS	
A. <i>Antibody-mediated glomerular injury</i>	
1. Immune-complex disease	Immune-complex mediated GN (Acute diffuse proliferative GN, membranous GN, membranoproliferative GN, IgA nephropathy; secondary glomerular disease in SLE, malaria etc.)
2. Anti-glomerular basement membrane (Anti-GBM) disease	Goodpasture's disease
3. Alternate pathway disease	Membranoproliferative GN type II
4. Other mechanisms (anti-neutrophil cytoplasmic antibodies ANCA, anti-endothelial cell antibodies AECA)	Focal segmental GS
B. <i>Cell-mediated glomerular injury</i>	Pauci-immune GN (type III RPGN)
C. <i>Secondary pathogenetic mechanisms</i>	Mediate glomerular injury in various primary and secondary glomerular diseases
II. NON-IMMUNOLOGIC MECHANISMS	
1. Metabolic	Diabetic nephropathy, Fabry's disease
2. Haemodynamic	Hypertensive nephrosclerosis, FSGS
3. Deposition	Amyloid nephropathy
4. Infectious	HIV-nephropathy, immune-complex GN in SABA
5. Drugs	NSAIDs-associated minimal change disease
6. Inherited	Alport's syndrome, nail-patella syndrome

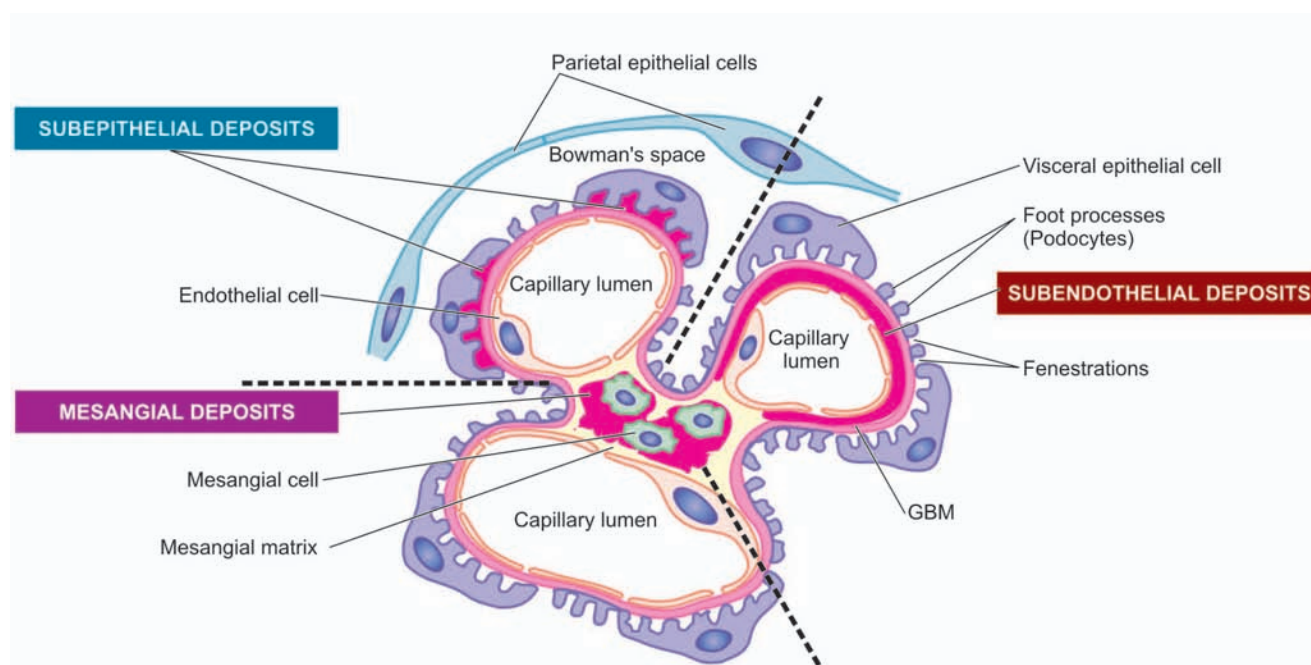


FIGURE 29.1

Electron microscopic appearance of a portion of glomerular lobule, showing three patterns of irregular or granular glomerular deposits in immune-complex disease.

a protein of group A streptococci), viral and parasitic products and drugs.

ii) Circulating immune complex deposits. This mechanism used to be considered very important for glomerular injury but now it is believed that circulating immune complexes cause glomerular damage under certain circumstances only. These situations are: their presence in high concentrations for prolonged periods, or when they possess special properties that cause their binding to glomeruli, or when host mechanisms are defective and fail to eliminate immune complexes. The antigens evoking antibody response may be endogenous (e.g. in SLE) or may be exogenous (e.g. Hepatitis B virus, *Treponema pallidum*, *Plasmodium falciparum* and various tumour antigens). The antigen-antibody complexes are formed in the circulation and then trapped in the glomeruli where they produce glomerular injury after combining with complement.

Immune complex GN is observed in the following human diseases:

i) **Primary GN** e.g. acute diffuse proliferative GN, membranous GN, membranoproliferative GN, IgA nephropathy and some cases of rapidly progressive GN and focal GN.

ii) **Systemic diseases** e.g. glomerular disease in SLE, malaria, syphilis, hepatitis, Henoch-Schonlein purpura and idiopathic mixed cryoglobulinaemia.

2. ANTI-GBM DISEASE. Less than 5% cases of human GN are associated with anti-GBM antibodies. The constituent of GBM acting as antigen appears to be a component of collagen IV of the basement membrane. The experimental model of anti-GBM disease is *Masugi nephritis* (nephrotoxic serum nephritis) produced in rats by injection of heterologous antibodies against GBM prepared in rabbits by immunisation with rat kidney tissue.

Anti-GBM disease is classically characterised by *interrupted linear deposits* of anti-GBM antibodies (mostly IgG; rarely IgA and IgM) and complement (mainly C3) along the glomerular basement membrane. These deposits are detected by immunofluorescence microscopy or by electron microscopy.

Anti-GBM disease is characteristically exemplified by glomerular injury in Goodpasture's syndrome in some cases of rapidly progressive GN. About half to two-third of the patients with renal lesions in Goodpasture's syndrome have pulmonary haemorrhage mediated by cross-reacting autoantibodies against alveolar basement membrane.

3. ALTERNATE PATHWAY DISEASE. As apparent from the above mechanisms, the complement system, in particular C3, contributes to glomerular injury in most forms of GN. Deposits of C3 are associated with the early components C1, C2 and C4 which are evidence of classic pathway activation of complement. But in alternate pathway activation, there is decreased serum C3 level, decreased serum levels of factor B and properdin, normal serum levels of C1, C2 and C4 but C3 and properdin are found deposited in the glomeruli without immunoglobulin deposits, reflecting activation of alternate pathway of complement. Such patients have circulating anti-complementary nephritic factor called C3NeF which is an IgG antibody and acts as an auto-antibody to the alternate C3 convertase and enhances alternate pathway activity.

The deposits in alternate pathway disease are characteristically electron-dense under electron microscopy, glomerular lesions in such cases are referred to as *dense-deposit disease*.

Alternate pathway disease occurs in most cases of type II membranoproliferative GN, some patients of rapidly progressive GN, acute diffuse proliferative GN, IgA nephropathy and in SLE.

4. OTHER MECHANISMS OF ANTIBODY-MEDIATED INJURY. A few autoantibodies have been implicated in some patients of focal segmental glomerulosclerosis and few other types of GN. These antibodies include the following:

i) Anti-neutrophil cytoplasmic antibodies (ANCA). About 40% cases of rapidly progressive GN are deficient in immunoglobulins in glomeruli and are positive for ANCA against neutrophil cytoplasmic antigens in their circulation. ANCA causes endothelial injury by generation of reactive oxygen radicals.

ii) Anti-endothelial cell antibodies (AECA). Auto-antibodies against endothelial antigens have been detected in circulation in several inflammatory vasculitis and glomerulonephritis. These antibodies increase the adhesiveness of leucocytes to endothelial cells.

B. Cell-mediated Glomerular Injury (Delayed-type Hypersensitivity)

Recent evidence suggests that cell-mediated immune reactions may be involved in causing glomerular injury, particularly in cases with deficient immunoglobulins (e.g. in pauci-immune type glomerulonephritis in RPGN). Cytokines and other mediators released by activated T cells stimulate cytotoxicity, recruitment of

more leucocytes and fibrogenesis. CD4+ T lymphocytes recruit more macrophages while CD8+ cytotoxic T lymphocytes and natural killer cells cause further glomerular cell injury by antibody-dependent cell toxicity. Soluble factor derived from T lymphocytes is implicated in proteinuria in minimal change disease and focal GS.

However, cell-mediated injury, is yet less clear than antibody-mediated glomerular injury.

C. Secondary Pathogenetic Mechanisms (Mediators of Immunologic Injury)

Secondary pathogenetic mechanisms are a number of mediators of immunologic glomerular injury operating in man and in experimental models. These include the following:

1. NEUTROPHILS. Neutrophils are conspicuous in certain forms of glomerular disease such as in acute diffuse proliferative GN, and may also be present in membranoproliferative GN and lupus nephritis. Neutrophils can mediate glomerular injury by activation of complement as well as by release of proteases, arachidonic acid metabolites and oxygen-derived free radicals. These agents cause degradation of GBM and cell injury.

2. MONONUCLEAR PHAGOCYTES. Many forms of human and experimental proliferative GN are associated with glomerular infiltration by monocytes and macrophages. Accumulation of mononuclear phagocytes is considered an important constituent of hypercellularity in these forms of GN aside from proliferation of mesangial and endothelial cells. Activated macrophages release a variety of biologically active substances which take part in glomerular injury.

3. COMPLEMENT SYSTEM. The pathogenetic role of classical and alternate pathway of activation of complement has already been highlighted above. Besides the components of complement which mediate glomerular injury via neutrophils already mentioned, C5b9 is capable of inducing damage to GBM directly.

4. PLATELETS. Platelet aggregation and release of mediators play a role in the evolution of some forms of GN. Increased intrarenal platelet consumption has been found to occur in some forms of glomerular disease.

5. MESANGIAL CELLS. There is evidence to suggest that mesangial cells present in the glomeruli may be stimulated to produce mediators of inflammation and take part in glomerular injury.

6. COAGULATION SYSTEM. The presence of fibrin in early crescents in certain forms of human and experimental GN suggests the role of coagulation system in glomerular damage. Fibrinogen may leak into Bowman's space and act as stimulus for cell proliferation. Crescents usually transform into scar tissue under the influence of fibronectin which is regularly present in crescents in human glomerular disease.

II. NON-IMMUNOLOGIC MECHANISMS

Though most forms of GN are mediated by immunologic mechanisms, a few examples of glomerular injury by non-immunologic mechanisms are found. These are:

1. *Metabolic glomerular injury* e.g. in diabetic nephropathy (due to hyperglycaemia), Fabry's disease (due to sulfatidosis).
2. *Haemodynamic glomerular injury* e.g. systemic hypertension, intraglomerular hypertension in FSGS.
3. *Deposition diseases* e.g. amyloidosis.
4. *Infectious diseases* e.g. HBV, HCV, HIV, *E. coli*-derived nephrotoxin.
5. *Drugs* e.g. minimal change disease due to NSAIDs.
6. *Inherited glomerular diseases* e.g. Alport's syndrome, nail-patella syndrome.

The evolution of end-stage renal failure is explained on the basis of adaptive glomerular hypertrophy of unaffected glomeruli that results in increased glomerular blood flow and increased glomerular capillary pressure inducing intraglomerular hypertension. These events lead to increased deposition of mesangial matrix and proliferation of mesangial cells, endothelial and epithelial cell injury, and eventually to progressive glomerulosclerosis and end-stage renal failure.

SPECIFIC TYPES OF GLOMERULAR DISEASES

Classification of different forms of glomerular diseases is already presented in Table 29.1. Features of some important types are described below while a summary of major forms of primary glomerulonephritis is given in Table 29.7 at the end of this discussion.

I. PRIMARY GLOMERULONEPHRITIS

Acute Glomerulonephritis

(Synonyms: *Acute Diffuse Proliferative GN*, *Diffuse Endocapillary GN*)

Acute GN is known to follow acute infection and characteristically presents as acute nephritic syndrome. Based on etiologic agent, acute GN is subdivided into 2 main

groups: acute post-streptococcal GN and acute non-streptococcal GN, the former being more common.

ACUTE POST-STREPTOCOCCAL GN

Acute post-streptococcal GN is fairly common form of GN, seen most commonly in children 6 to 16 years of age but adolescents and adults may also be affected. The onset of disease is generally sudden after 1-2 weeks of streptococcal infection, most frequently of the throat (e.g. streptococcal pharyngitis) and sometimes of the skin (e.g. streptococcal impetigo).

ETIOPATHOGENESIS. The relationship between streptococcal infection and this form of GN is now well established. Particularly nephritogenic are types 12,4,1 and Red Lake of group A β -haemolytic streptococci (compare the etiologic agent with that of RHD, page 317). The glomerular lesions appear to result from deposition of immune complexes in the glomeruli. The evidences cited in support are as under:

- i) There is *epidemiological* evidence of preceding streptococcal sore throat or skin infection about 1-2 weeks prior to the attack.
- ii) The *latent period* between streptococcal infection and onset of clinical manifestations of the disease is compatible with the period required for building up of antibodies.
- iii) Streptococcal infection may be identified by *culture* or may be inferred from elevated titres of *antibodies* against streptococcal antigens. These include:
 - anti-streptolysin O (ASO);
 - anti-deoxyribonuclease B (anti-DNAse B);
 - anti-streptokinase (ASKase);
 - anti-nicotinyl adenine dinucleotidase (anti-NADase); and
 - anti-hyaluronidase (AHase).
- iv) There is usually *hypocomplementaemia* indicating involvement of complement in the glomerular deposits.
- v) More recently, it has been possible to identify antigenic component of streptococci which is cytoplasmic antigen, *endostreptosin*.

PATHOLOGIC CHANGES. *Grossly*, the kidneys are symmetrically enlarged, weighing one and a half to twice the normal weight. The cortical as well as sectioned surface show petechial haemorrhages giving the characteristic appearance of flea-bitten kidney.

Light microscopic findings are as under:

i) Glomeruli—The glomeruli are affected diffusely. They are enlarged and hypercellular. The diffuse hypercellularity of the tuft is due to proliferation of mesangial, endothelial and occasionally epithelial cells (*acute proliferative lesions*) as well as by infiltration of leucocytes, chiefly polymorphs and sometimes monocytes (*acute exudative lesion*, Fig. 29.2,A). There may be small deposits of fibrin within the capillary lumina and in the mesangium.

ii) Tubules—Tubular changes are not very striking. There may be swelling and hyaline droplets in tubular cells, and tubular lumina may contain red cell casts.

iii) Interstitium—There may be some degree of interstitial oedema and leucocytic infiltration.

iv) Vessels—Changes in arteries and arterioles are seldom present in acute GN.

Electron microscopic findings, aside from confirming the light microscopic findings, are the characteristic electron-dense irregular deposits ('*humps*') on the epithelial side of the GBM. These deposits represent the immune complexes (Fig. 29.2,B).

Immunofluorescence microscopy reveals that the irregular deposits along the GBM consist principally of IgG and complement C3.

CLINICAL FEATURES. Typically, the patient is a young child, presenting with acute nephritic syndrome (page 191), having sudden and abrupt onset following an

episode of sore throat 1-2 weeks prior to the development of symptoms. The features include microscopic or intermittent haematuria, red cell casts, mild non-selective proteinuria (less than 3 gm per 24 hrs), hypertension, periorbital oedema and variably oliguria. Less often, the presentation may be as nephrotic syndrome. In adults, the features are atypical and include sudden hypertension, oedema and azotaemia. Development of hypertension in either case is a poor prognostic sign.

Prognosis varies with the age of the patient. Children almost always (95%) recover completely with reversal of proliferative glomerular changes. Complications arise more often in adults and occasionally in children. These include development of rapidly progressive GN, chronic GN, uraemia and chronic renal failure.

ACUTE NON-STREPTOCOCCAL GN

About one-third cases of acute GN are caused by organisms other than haemolytic streptococci. These include other bacteria (e.g. staphylococci, pneumococci, meningococci, *Salmonella* and *Pseudomonas*), viruses (e.g. hepatitis B virus, mumps, infectious mononucleosis and varicella), parasitic infections (e.g. malaria, toxoplasmosis and schistosomiasis) and syphilis. The appearance of renal biopsy by light microscopy, EM and immunofluorescence microscopy is similar to that seen in acute post-streptococcal GN. The prognosis of non-streptococcal GN is not as good as that of streptococcal GN.

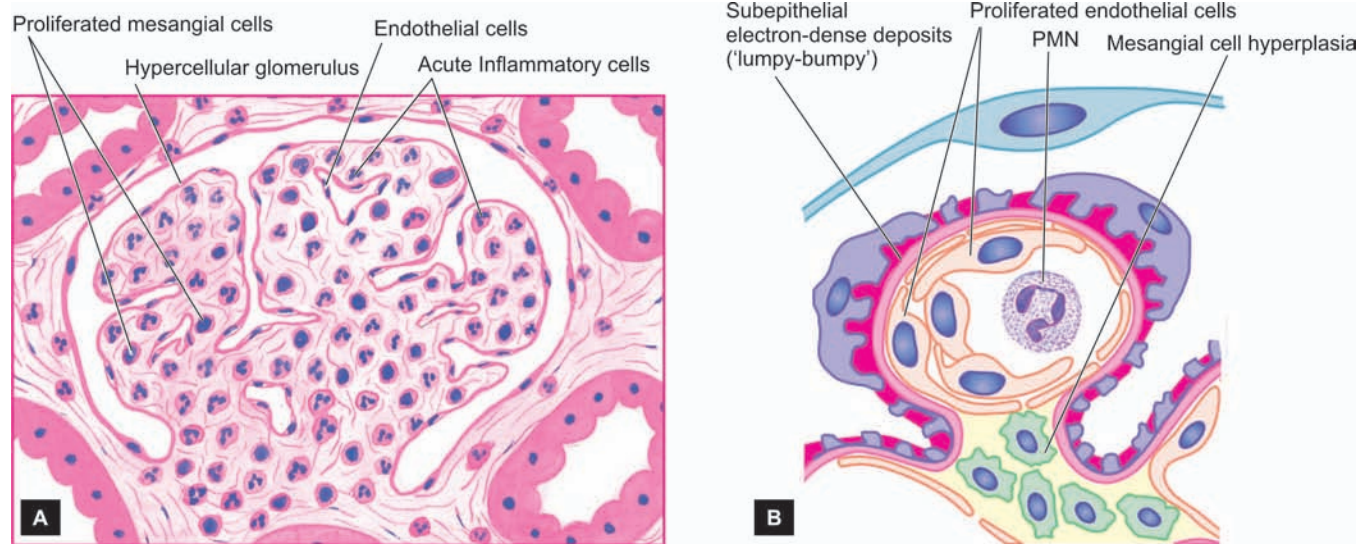


FIGURE 29.2

Acute post-streptococcal GN. A, light microscopic appearance. There is increased cellularity due to proliferation of mesangial cells, endothelial cells and some epithelial cells and infiltration of the tuft by neutrophils and monocytes. B, Electron microscopic appearance of a portion of glomerular lobule, showing characteristic electron-dense irregular deposits or '*humps*' on the epithelial side of the GBM.

Rapidly Progressive Glomerulonephritis (Synonyms; RPGN, Crescentic GN, Extracapillary GN)

RPGN presents with an acute reduction in renal function resulting in acute renal failure in a few weeks or months. It is characterised by formation of 'crescents' (*crescentic GN*) outside the glomerular capillaries (*extracapillary GN*). 'Crescents' are formed from the proliferation of parietal epithelial cells lining Bowman's capsule with contribution from visceral epithelial cells and the invading mononuclear cells. The stimulus for crescent formation appears to be the presence of fibrin in the capsular space. RPGN occurs most frequently in adults, with a slight male preponderance. Prognosis of RPGN in general is dismal.

ETIOPATHOGENESIS. A number of primary glomerular and systemic diseases are characterised by formation of crescents. Based on the etiologic agents and pathogenetic mechanism, patients with RPGN are divided into 3 groups (Table 29.6):

- RPGN in systemic diseases (anti-GBM type);
- post-infectious RPGN (immune-complex type); and
- pauci-immune RPGN.

There are three main serologic markers in RPGN:

- i) serum C3 level,
- ii) anti-GBM antibody; and
- iii) anti-neutrophil cytoplasmic antibody (ANCA)

Type I RPGN: Anti-GBM disease. A number of systemic diseases such as Goodpasture's syndrome, SLE, vasculitis, Wegener's granulomatosis, Henoch-Schonlein purpura and idiopathic mixed cryoglobulinaemia are associated with crescentic GN. Goodpasture's syndrome

is the characteristic example of anti-GBM disease and is described below:

Goodpasture's syndrome. Goodpasture's syndrome is characterised by acute renal failure due to RPGN and pulmonary haemorrhages. The condition is more common in males in 3rd decade of life. The disease results from damage to the glomeruli by anti-GBM antibodies which cross-react with alveolar basement membrane and hence, produce renal as well as pulmonary lesions. The evidences in support are the characteristic linear deposits of anti-GBM antibodies consisting of IgG and complement along the GBM, detection of circulating anti-GBM antibodies and induction of glomerular lesions with injection of anti-GBM antibodies experimentally in monkeys. Pulmonary lesions can be experimentally induced if the lungs are previously injured by viral or bacterial infection or exposed to hydrocarbons. The Goodpasture's antigen appears to be a component of collagen type IV.

Type II RPGN: Immune complex disease. A small proportion of cases of post-streptococcal GN, particularly in adults and sometimes of non-streptococcal origin, develop RPGN. The evidences in support of post-infectious RPGN having immune complex pathogenesis are granular deposits of immune complexes of IgG and C3 along the glomerular capillary walls, lowering of blood complement levels and demonstration of circulating complexes.

Type III RPGN: Pauci-immune GN. These include cases of Wegener's granulomatosis and microscopic polyarteritis nodosa. The pathogenesis of pauci-immune GN is yet not fully defined. However, majority of these

TABLE 29.6: Distinguishing Features of Three Main Categories of Rapidly Progressive Glomerulonephritis.

FEATURE	TYPE I RPGN (ANTI-GBM DISEASE)	TYPE II RPGN (IMMUNE COMPLEX DISEASE)	TYPE III RPGN (PAUCI-IMMUNE GN)
1. <i>Clinical syndrome</i>	Nephritic	Nephritic	Nephritic
2. <i>Pathogenetic type</i>	Anti-GBM	Immune-complex	Pauci-immune
3. <i>Immunofluorescence</i>	Linear Ig and C3	Granular Ig and C3	Sparse or absent Ig and C3
4. <i>Serologic markers</i>			
i) <i>Serum C3 level</i>	Normal	Low-to-normal	Normal
ii) <i>Anti-GBM antibody</i>	Positive	Negative	Negative
iii) <i>ANCA</i>	Negative	Negative	Positive
5. <i>Underlying cause</i>	Idiopathic Goodpasture's syndrome, SLE, vasculitis, Wegener's granulomatosis, Henoch-Schonlein purpura	Idiopathic Post-infectious (post-streptococcal GN)	Idiopathic Polyarteritis nodosa, Wegener's granulomatosis

patients are ANCA-positive, implying a defect in humoral immunity. Serum complement levels are normal and anti-GBM antibody is negative. There is little or no glomerular immune deposit (i.e. pauci-immune).

PATHOLOGIC CHANGES. *Grossly*, the kidneys are usually enlarged and pale with smooth outer surface (*large white kidney*). Cut surface shows pale cortex and congested medulla.

Light Microscopic findings vary according to the cause but in general following features are present:

i) Glomeruli—Irrespective of the underlying etiology, all forms of RPGN show pathognomonic ‘crescents’ on the inside of Bowman’s capsules. These are collections of pale-staining polygonal cells which commonly tend to be elongated. Eventually, crescents obliterate the Bowman’s space and compress the glomerular tuft. Fibrin deposition is invariably present alongside crescents. Besides the crescents, glomerular tufts may show increased cellularity as a result of proliferation of endothelial and mesangial cells and leucocytic infiltration (Fig. 29.3, A). Fibrin thrombi are frequently present in the glomerular tufts.

ii) Tubules—Tubular epithelial cells may show hyaline droplets. Tubular lumina may contain casts, red blood cells and fibrin.

iii) Interstitium—The interstitium is oedematous and may show early fibrosis. Inflammatory cells, usually lymphocytes and plasma cells, are commonly distributed in the interstitial tissue.

iv) Vessels—Arteries and arterioles may show no change, but cases associated with hypertension usually show severe vascular changes.

Electron microscopic findings vary according to the type of RPGN. Post-infectious RPGN cases show electron-dense subepithelial granular deposits similar to those seen in acute GN, while cases of RPGN in Goodpasture’s syndrome show characteristic linear deposits along the GBM (Fig. 29.3, B).

Immunofluorescence microscopy shows:

- linear pattern of RPGN in Goodpasture’s syndrome (type I RPGN), containing IgG accompanied by C3 along the capillaries
- granular pattern of post-infectious RPGN (type II RPGN) consisting of IgG and C3 along the capillary wall; and
- scanty or no deposits of immunoglobulin and C3 in pauci-immune GN (type III RPGN).

CLINICAL FEATURES. Generally, the features of post-infectious RPGN are similar to those of acute GN, presenting as acute renal failure. The patients of

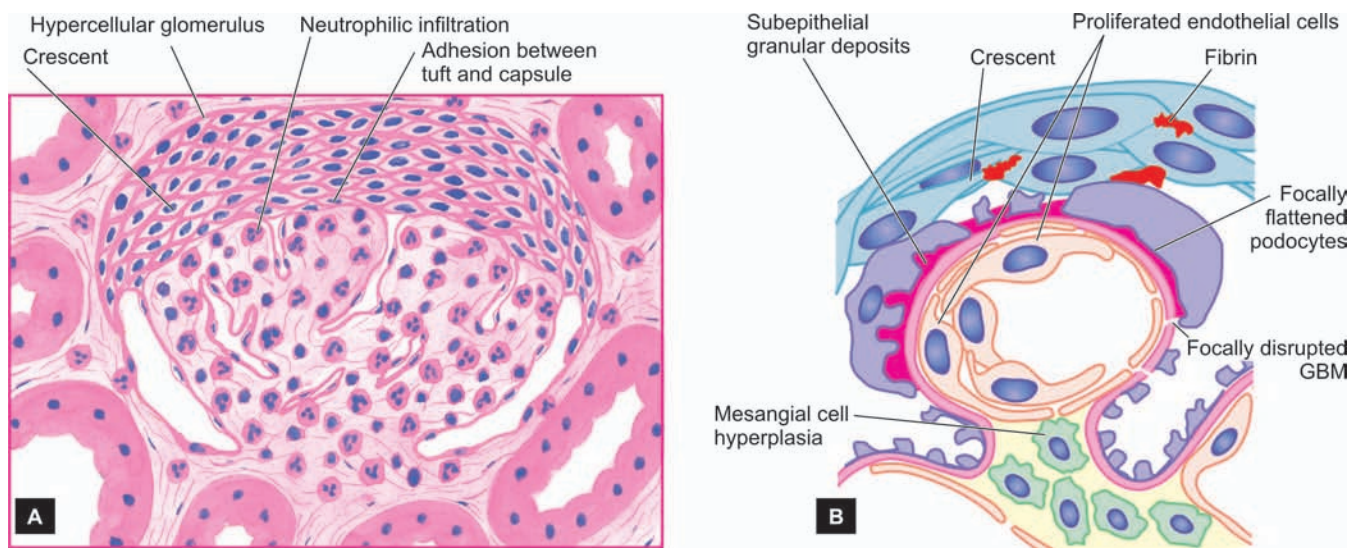


FIGURE 29.3

A, Post-infectious RPGN, light microscopic appearance. There are crescents in Bowman’s space forming adhesions between the glomerular tuft and Bowman’s capsule. The tuft shows hypercellularity and leucocytic infiltration. B, Electron microscopic appearance of a portion of glomerular lobule, showing epithelial crescent formation and subepithelial granular deposits.

Goodpasture's syndrome may present as acute renal failure and/or associated intrapulmonary haemorrhage producing recurrent haemoptysis. Prognosis of all forms of RPGN is poor. However, post-infectious cases have somewhat better outcome and may show recovery.

Minimal Change Disease

(Synonyms: MCD, Lipoid Nephrosis, Foot Process Disease, Nil Deposit Disease)

Minimal change disease (MCD) is a condition in which the nephrotic syndrome is accompanied by no apparent change in glomeruli by light microscopy. Its other synonyms, lipoid nephrosis and foot process disease, are descriptive terms for fatty changes in the tubules and electron microscopic appearance of flattened podocytes respectively. Minimal change disease accounts for 80% cases of nephrotic syndrome in children under 16 years of age with preponderance in boys (ratio of boys to girls 2:1).

ETIOPATHOGENESIS. The etiology of MCD remain elusive. However, following two groups have been identified:

- i) Idiopathic (majority of cases).
- ii) Cases associated with systemic diseases (Hodgkin's disease, HIV infection) and drug therapy (e.g. NSAIDs, rifampicin, interferon- α).

The following features point to possible immunologic pathogenesis for MCD:

- i) Absence of deposits by immunofluorescence.
- ii) Normal circulating levels of complement but presence of circulating immune complexes in many cases.
- iii) Universal satisfactory response to steroid therapy.
- iv) Evidence of increased suppressor T cell activity with elaboration of cytokines (interleukin-8, tumour necrosis factor) which probably cause foot process flattening.
- v) Recent detection of a mutation in nephrin gene in cases of congenital MCD has focused attention on genetic basis.

The nephrotic syndrome in MCD in children is characterised by *selective proteinuria* containing mainly albumin, and minimal amounts of high molecular weight proteins such as α 2-macroglobulin. The basis for selective proteinuria appears to be:

- i) reduction of normal negative charge on GBM due to loss of heparan sulfate proteoglycan from the GBM; and
- ii) change in the shape of epithelial cells producing foot process flattening due to reduction of sialoglycoprotein cell coat.

Adults having MCD, however, have *non-selective proteinuria*, suggesting more extensive membrane permeability defect.

PATHOLOGIC CHANGES. *Grossly*, the kidneys are of normal size and shape.

By light microscopy, the findings are as under:

- i) **Glomeruli**—The most characteristic feature is no apparent abnormality in the glomeruli except for slight increase in the mesangial matrix at the most.
- ii) **Tubules**—There is presence of fine lipid vacuolation and hyaline droplets in the cells of proximal convoluted tubules and, hence, the older name of the condition as 'lipoid nephrosis'.
- iii) **Interstitium**—There may be oedema of the interstitium.
- iv) **Vessels**—Blood vessels do not show any significant change (minimal change).

By electron microscopy, the most characteristic feature of the disease is identified which is diffuse flattening of foot processes of the visceral epithelial cells (podocytes) and, hence, the name foot process disease (Fig. 29.4). Unlike other forms of GN, no deposits are seen and the GBM is normal.

By immunofluorescence microscopy, no deposits of complement or immunoglobulins are recognised (nil deposit disease).

CLINICAL FEATURES. The classical presentation of MCD is of fully-developed nephrotic syndrome with massive and *highly selective proteinuria*, but hypertension is unusual. Most frequently, the patients are children under 16 years (peak incidence at 6-8 years of age).

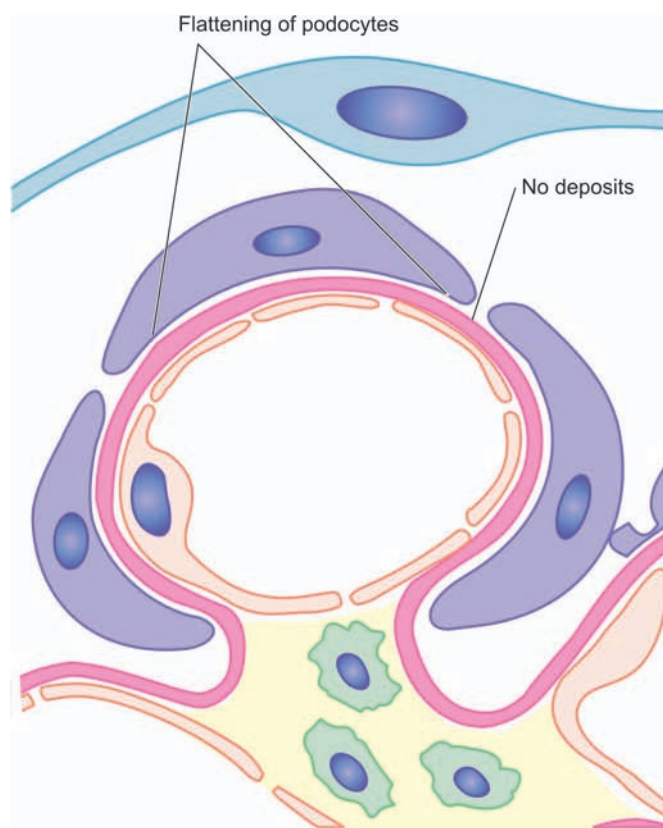
The onset may be preceded by an upper respiratory infection, atopic allergy or immunisation.

The disease characteristically responds to steroid therapy. In spite of remissions and relapses, long-term prognosis is very good and most children become free of albuminuria after several years.

Membranous Glomerulonephritis

(Synonym: Epimembranous Nephropathy)

Membranous GN is characterised by wide-spread thickening of the glomerular capillary wall and is the most common cause of nephrotic syndrome in adults. In about 15% of cases, membranous GN is *secondary* to an underlying condition (e.g. SLE, malignancies, infections such as chronic hepatitis B and C, syphilis, malaria and drugs), while in the majority of cases (85%) it is truly *idiopathic*.

**FIGURE 29.4**

Minimal change disease, EM appearance of a portion of glomerular lobule showing diffuse fusion or flattening of foot processes of visceral epithelial cells (podocytes). The GBM is normal and there are no deposits.

ETIOPATHOGENESIS. *Idiopathic membranous GN* is an immune complex disease. The deposits of immune complex are formed locally because circulating immune complexes are detected in less than a quarter of cases. Since leucocytic infiltration is not a feature of membranous GN, damage to the GBM is mediated directly by complement. While nephritogenic antigen against which autoantibodies are formed in *idiopathic membranous GN* is not known yet, the antigen in cases of *secondary membranous GN* is either an endogenous (e.g. DNA in SLE) or exogenous one (e.g. hepatitis B virus, tumour antigen, treponema antigen, drug therapy with penicillamine).

PATHOLOGIC CHANGES. *Grossly*, the kidneys are enlarged, pale and smooth.

Light microscopy shows the following findings:

i) Glomeruli—The characteristic finding is diffuse thickening of the glomerular capillary walls with all

the glomeruli being affected more or less uniformly. As the disease progresses, the deposits are incorporated into enormously thickened basement membrane, producing 'duplication' of GBM which is actually formation of a new basement membrane. These basement membrane changes are best appreciated by silver impregnation stains (black colour) or by periodic acid-Schiff stain (pink colour). There is no cellular proliferation in the glomerular tufts (Fig. 29.5,A).

ii) Tubules—The renal tubules remain normal except in the early stage when lipid vacuolation of the proximal convoluted tubules may be seen.

iii) Interstitium—The interstitium may show fine fibrosis and scanty chronic inflammatory cells.

iv) Vessels—In the early stage, vascular changes are not prominent, while later hypertensive changes of arterioles may occur.

Electron microscopy shows electron-dense deposits situated in the epithelial side of the GBM. The basement membrane material protrudes between deposits as 'spikes' (Fig. 29.5,B).

Immunofluorescence microscopy reveals granular deposits of immune complexes consisting of IgG associated with complement C3. In secondary cases of membranous GN the relevant antigen such as hepatitis B or tumour antigen may be seen.

CLINICAL FEATURES. The presentation in majority of cases is insidious onset of nephrotic syndrome in an adult. The proteinuria is usually of *non-selective* type. In addition, microscopic haematuria and hypertension may be present at the onset or may develop during the course of the disease. The changes in membranous GN are irreversible in majority of patients. Progression to impaired renal function and end-stage renal disease with progressive azotaemia occurs in approximately 50% cases within a span of 2 to 20 years. Renal vein thrombosis has been found to develop in patients with membranous GN due to hypercoagulability. The role and beneficial effects of steroid therapy with or without the addition of immunosuppressive drugs is debatable.

Membranoproliferative Glomerulonephritis (Synonyms: MPGN, Mesangiocapillary GN)

Membranoproliferative GN is another important cause of nephrotic syndrome in children and young adults. As the name implies, it is characterised by two histologic features—increase in cellularity of the mesangium associated with increased lobulation of the tuft, and irregular thickening of the capillary wall.

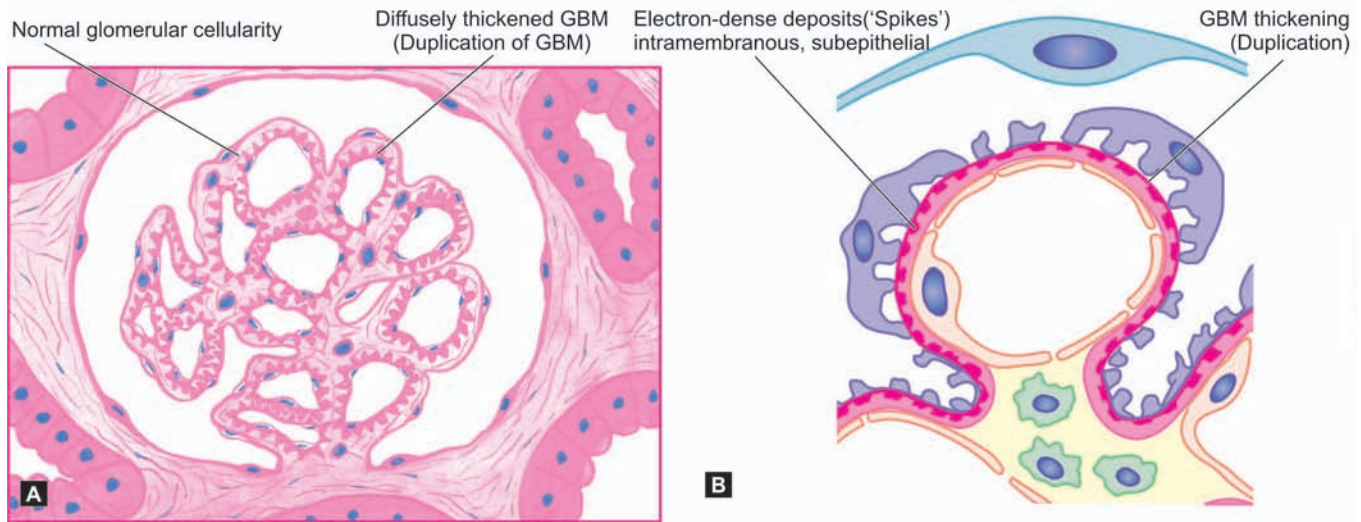


FIGURE 29.5

Membranous GN. A, Light microscopic appearance, showing normal glomerular cellularity but the capillary walls are diffusely thickened due to duplication of the GBM. B, Electron microscopic appearance of a portion of glomerular lobule. There are subepithelial deposits of electron-dense material so that the basement membrane material protrudes between these deposits.

ETIOPATHOGENESIS. Etiology of MPGN is unknown though in some cases there is evidence of preceding streptococcal infection. Based on ultrastructural, immunofluorescence and pathogenetic mechanisms, three types of MPGN are recognised:

■ **Type I or classic form** is an example of immune complex disease and comprises more than 70% cases. It is characterised by immune deposits in the subendothelial position. Immune-complex MPGN is seen in association with systemic immune-complex diseases (e.g. SLE, mixed cryoglobulinaemia, Sjögren's syndrome), chronic infections (e.g. bacterial endocarditis, HIV, hepatitis B and C) and malignancies (e.g. lymphomas and leukaemias).

■ **Type II or dense deposit disease** is the example of alternate pathway disease (page 378) and constitutes about 30% cases. The capillary wall thickening is due to the deposition of electron-dense material in the lamina densa of the GBM. Type II MPGN is an autoimmune disease in which patients have IgG autoantibody termed C3 nephritic factor. Type II cases have an association with partial lipodystrophy, an unusual condition of unknown pathogenesis characterised by symmetrical loss of subcutaneous fat from the upper half of the body.

■ **Type III** is rare and shows features of type I MPGN and membranous nephropathy in association with systemic diseases or drugs.

PATHOLOGIC CHANGES. Grossly and by light microscopy, all the three types of MPGN are similar.

Grossly, the kidneys are usually pale in appearance and firm in consistency.

By light microscopy, the features are as under:

i) **Glomeruli**—Glomeruli show highly characteristic changes. They are enlarged with accentuated lobular pattern. The enlargement is due to variable degree of mesangial cellular proliferation and increase in mesangial matrix. The GBM is considerably thickened, which with silver stains shows two basement membranes with a clear zone between them. This is commonly referred to as 'double contour', *splitting*, or 'tram track' appearance (Fig. 29.6,A).

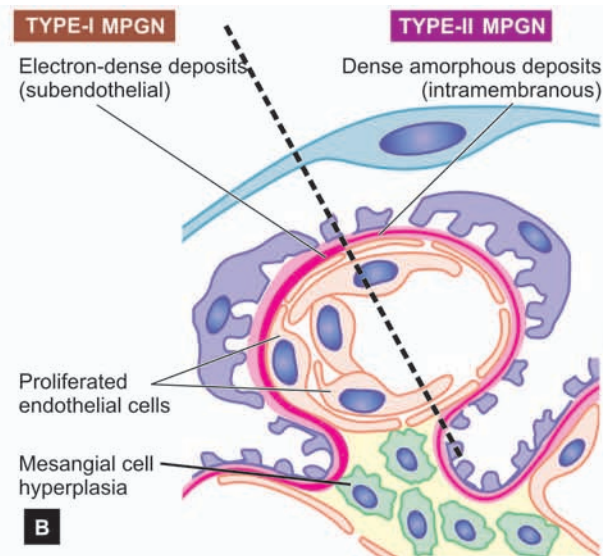
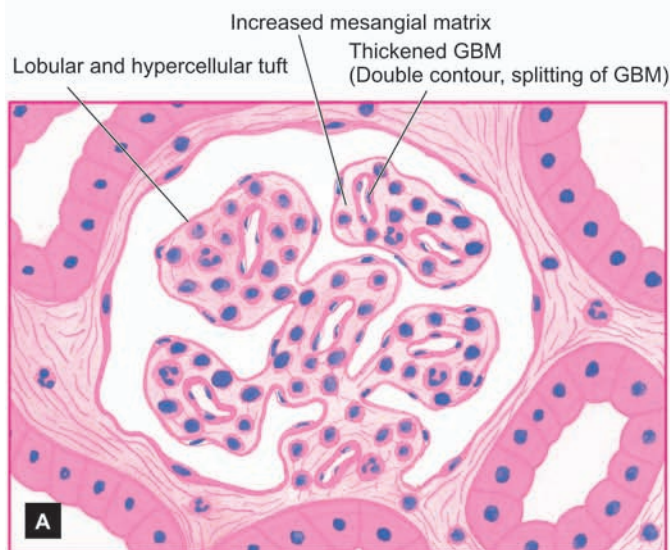
ii) **Tubules**—Tubular cells may show vacuolation and hyaline droplets.

iii) **Interstitium**—There may be scattered chronic inflammatory cells and some finely granular foam cells in the interstitium.

iv) **Vessels**—Vascular changes are prominent in cases in which hypertension develops.

By electron microscopy and immunofluorescence microscopy, the changes are different in the three types of MPGN (Fig. 29.6,B):

Type I: It shows *electron-dense deposits* in subendothelial location conforming to immune-complex character of the disease. These deposits reveal posi-

**FIGURE 29.6**

Membranoproliferative GN. A, Light microscopic appearance. The glomerular tufts show lobulation and mesangial hypercellularity. There is increase in the mesangial matrix between the capillaries. There is widespread thickening of the GBM. B, Electron microscopic appearance of a portion of glomerular lobule showing features of type I (left half) and type II (right half) MPGN. Type I (classic form) shows the characteristic subendothelial electron-dense deposits, while type II (dense deposit disease) is characterised by intramembranous dense deposits.

tive fluorescence for C3 and slightly fainter staining for IgG.

Type II: The hallmark of type II MPGN is the presence of *dense amorphous deposits* within the lamina densa of the GBM and in the mesangium. Immunofluorescence studies reveal the universal presence of C3 and properdin in the deposits but the immunoglobulins are usually absent.

Type III: This rare form has *electron-dense deposits* within the GBM as well as in subendothelial and subepithelial regions of the GBM. Immunofluorescence studies show the presence of C3, IgG and IgM.

CLINICAL FEATURES. Clinically, there are many similarities between the main forms of MPGN. The most common age at diagnosis is between 15 and 20 years. Approximately 50% of the patients present with nephrotic syndrome; about 30% have asymptomatic proteinuria; and 20% have nephritic syndrome at presentation. The proteinuria is non-selective. Haematuria and hypertension are frequently present. Hypocomplementaemia is a common feature. With time, majority of patients progress to renal failure, while some continue to have proteinuria, haematuria and hypertension with stable renal function.

Prognosis of type I is relatively better and majority of patients survive without clinically significant

impairment of GFR, while type II cases run a variable clinical course.

Chronic Glomerulonephritis (Synonym: End-Stage Kidney)

Chronic GN is the final stage of a variety of glomerular diseases which result in irreversible impairment of renal function. The conditions which may progress to chronic GN, in descending order of frequency, are as under:

- i) Rapidly progressive GN (90%)
- ii) Membranous GN (50%)
- iii) Membranoproliferative GN (50%)
- iv) Focal segmental glomerulosclerosis (50%)
- v) IgA nephropathy (40%)
- vi) Acute post-streptococcal GN (1%)

However, about 20% cases of chronic GN are *idiopathic* without evidence of preceding GN of any type.

PATHOLOGIC CHANGES. *Grossly*, the kidneys are usually small and contracted weighing as low as 50 gm each. The capsule is adherent to the cortex. The cortical surface is generally diffusely granular (Fig. 29.7,A). On cut section, the cortex is narrow and atrophic, while the medulla is unremarkable.

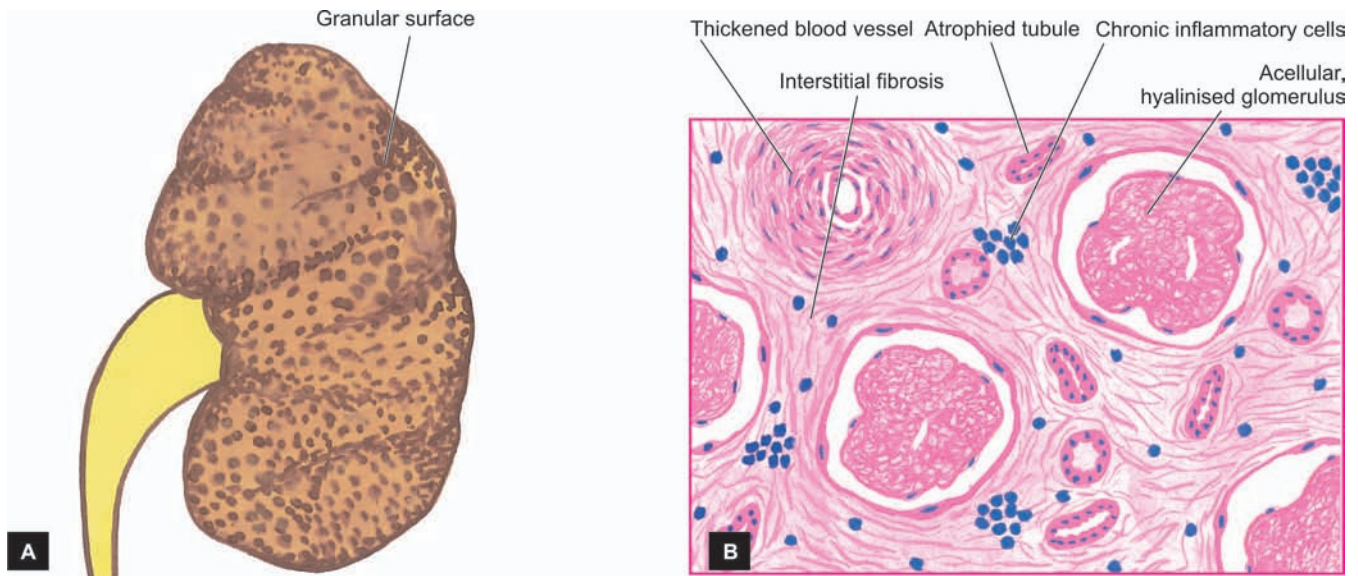


FIGURE 29.7

End-stage kidney (Chronic glomerulonephritis). A, Gross appearance showing diffusely granular cortical surface. B, Light microscopy shows acellular and completely hyalinised glomerular tufts, a hyalinised and thickened blood vessel and fine interstitial fibrosis with a few chronic inflammatory cells.

Microscopically, the changes vary greatly depending upon the underlying glomerular disease. In general, the following changes are seen (Fig. 29.7,B).

i) Glomeruli—Glomeruli are reduced in number and most of those present show completely hyalinised tufts, giving the appearance of acellular, eosinophilic masses which are PAS-positive. Evidence of underlying glomerular disease may be present.

ii) Tubules—Many tubules completely disappear and there may be atrophy of tubules close to scarred glomeruli. Tubular cells show hyaline-droplets, degeneration and tubular lumina frequently contain eosinophilic, homogeneous casts.

iii) Interstitium—There is fine and delicate fibrosis of the interstitial tissue and varying number of chronic inflammatory cells are often seen.

iv) Vessels—Advanced cases which are frequently associated with hypertension show conspicuous arterial and arteriolar sclerosis.

Patients of end-stage kidney disease on dialysis show a variety of dialysis associated changes that include acquired cystic disease, occurrence of adenomas and adenocarcinomas of the kidney, calcification of tufts and deposition of calcium oxalate crystals in tubules.

CLINICAL FEATURES. The patients are usually adults. The terminal stage of chronic GN is characterised by hypertension, uraemia and progressive deterioration of renal function. Besides the primary changes due to chronic renal failure, there are a variety of systemic manifestations of uraemia. These patients eventually die if they do not receive a renal transplant.

The salient features of various types of primary glomerulonephritis are summarised in Table 29.7.

PYELONEPHRITIS

The term tubulointerstitial nephritis is used for inflammatory process that predominantly involves the renal interstitial tissue and is usually accompanied by some degree of tubular damage. A number of bacterial and non-bacterial, acute and chronic conditions, may produce tubulointerstitial nephritis. The important and common types are acute and chronic pyelonephritis.

Acute Pyelonephritis

Acute pyelonephritis is an acute suppurative inflammation of the kidney caused by pyogenic bacteria.

ETIOPATHOGENESIS. Most cases of acute pyelonephritis follow infection of the lower urinary tract.

TABLE 29.7: Comparative Features of Major Forms of Primary Glomerulonephritis.

TYPE	CLINICAL FEATURES	PATHOGENESIS	PATHOLOGY		
			LM	EM	IFM
1. <i>Acute GN</i>	Acute nephrotic syndrome	Immune complex disease (local or circulating)	Diffuse proliferation, leucocytic infiltration	Subepithelial deposits ('humps')	Irregular IgG, C3
2. <i>RPGN</i>	Acute renal failure	i) Type I: anti-GBM type ii) Type II: immune complex type iii) Type III: pauci-immune RPGN	Proliferation, crescents	i) Linear deposits along GBM ii) Subepithelial deposits iii) No deposits	i) Linear IgG, C3 ii) Granular IgG, C3 iii) Negative
3. <i>Minimal change disease</i>	Nephrotic syndrome (highly selective proteinuria)	Reduction of normal negative charge on GBM ?Cell-mediated mechanism	Normal glomeruli, lipid vacuolation in tubules	Loss of foot processes, no deposits	Negative
4. <i>Membranous GN</i>	Nephrotic syndrome	Immune complex disease (local)	Diffuse thickening of capillary wall	Subepithelial deposits ('spikes')	Granular IgG, C3
5. <i>Membrano-proliferative GN</i>	Nephrotic syndrome	Type I: immune complex disease Type II: dense deposit disease (alternate pathway activation) Type III: rare, with systemic diseases and drugs	Lobular proliferation of mesangial cells, increased mesangial matrix, double contour of GBM	Type I: Subendothelial deposits Type II: Dense intramembranous deposits Type III: Subendothelial and subepithelial deposits	Type I: IgG, C3 Type II: C3 properdin Type III: C3 IgG, IgM
6. <i>Focal GN</i>	Variable, haematuria common	Variable, possibly immune complex disease	Focal and segmental proliferation	Mesangial deposits	IgA ± IgG, C3 and fibrin
7. <i>Focal Segmental glomerulosclerosis</i>	Nephrotic syndrome	i) Idiopathic ii) With superimposed primary glomerular disease iii) Secondary type	Focal and segmental sclerosis and hyalinosis	Loss of foot processes, electron dense deposits in regions of sclerosis and hyalinosis	IgM, C3
8. <i>IgA nephropathy</i>	Recurrent haematuria, mild proteinuria	Unknown, possibly alternate pathway disease	Variable, commonly focal proliferative GN	Mesangial electron-dense deposits	IgA ± IgG, C3, properdin
9. <i>Chronic GN</i>	Chronic renal failure	Variable	Hyalinised glomeruli	Variable	Variable

GN = glomerulonephritis; LM = light microscopy; EM = electron microscopy; IFM = immunofluorescence microscopy.

The most common pathogenic organism in urinary tract infection (UTI) is *Escherichia coli* (in 90% of cases), followed in decreasing frequency, by *Enterobacter*, *Klebsiella*, *Pseudomonas* and *Proteus*. The bacteria gain entry into the urinary tract, and thence into the kidney by one of the two routes: ascending infection and haematogenous infection (Fig. 29.8):

1. Ascending infection. This is the most common route of infection. The common pathogenic organisms are inhabitants of the colon and may cause faecal contamination of the urethral orifice, especially in females in reproductive age group. This has been variously attributed to shorter urethra in females liable to faecal contamination, hormonal influences facilitating bacterial

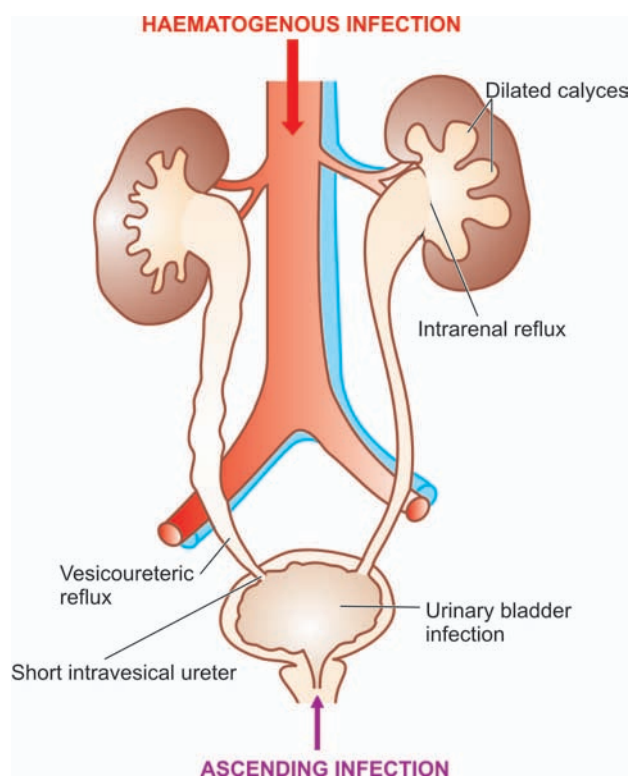


FIGURE 29.8

Pathogenesis of reflux nephropathy.

adherence to the mucosa, absence of prostatic secretions which have antibacterial properties, and urethral trauma during sexual intercourse. The last named produces what is appropriately labelled as '*honeymoon pyelitis*'. Ascending infection may occur in a normal individual but the susceptibility is increased in patients with diabetes mellitus, pregnancy, urinary tract obstruction or instrumentation. Bacteria multiply in the urinary bladder and produce asymptomatic bacteriuria found in many of these cases. After having caused urethritis and cystitis, the bacteria in a small proportion of cases ascend further up into the ureters against the flow of urine, extend into the renal pelvis and then the renal cortex. The role of vesico-ureteral reflux is primarily of importance in the pathogenesis of chronic pyelonephritis but not in acute pyelonephritis.

2. Haematogenous infection. Less often, acute pyelonephritis may result from blood-borne spread of infection. This occurs more often in patients with obstructive lesions in the urinary tract, and in debilitated or immunosuppressed patients.

PATHOLOGIC CHANGES. *Grossly*, well-developed cases of acute pyelonephritis show enlarged and swollen kidney that bulges on section. The cut surface shows small, yellow-white abscesses with a haemorrhagic rim. These abscesses may be several millimetres across and are situated mainly in the cortex. *Microscopically*, acute pyelonephritis is characterised by extensive acute inflammation involving the interstitium and causing destruction of the tubules. Generally, the glomeruli and renal blood vessels show considerable resistance to infection and are spared. The acute inflammation may be in the form of large number of neutrophils in the interstitial tissue and bursting into tubules, or may form focal neutrophilic abscesses in the renal parenchyma.

CLINICAL FEATURES. Classically, acute pyelonephritis has an acute onset with chills, fever, loin pain, lumbar tenderness, dysuria and frequency of micturition. Urine will show bacteria in excess of 100,000/ml, pus cells and pus cell casts in the urinary sediment. Institution of specific antibiotics, after identification of bacteria by culture followed by sensitivity test, eradicates the infection in majority of patients.

COMPLICATIONS. Complications of acute pyelonephritis are encountered more often in patients with diabetes mellitus or with urinary tract obstruction. There are 3 important complications of acute pyelonephritis. These are as under:

1. Papillary necrosis. Papillary necrosis or necrotising papillitis develops more commonly in analgesic abuse nephropathy and in sickle cell disease but may occur as a complication of acute pyelonephritis as well. It may affect one or both kidneys.

Grossly, the necrotic papillae are yellow to grey-white, sharply-defined areas with congested border and resemble infarction. The pelvis may be dilated. *Microscopically*, necrotic tissue is separated from the viable tissue by a dense zone of polymorphs. The necrotic area shows characteristic coagulative necrosis as seen in renal infarcts.

2. Pyonephrosis. Rarely, the abscesses in the kidney in acute pyelonephritis are extensive, particularly in cases with obstruction. This results in inability of the abscesses to drain and this transforms the kidney into a multilocular sac filled with pus called as pyonephrosis or renal carbuncle.

3. Perinephric abscess. The abscesses in the kidney may extend through the capsule of the kidney into the perinephric tissue and form perinephric abscess.

Chronic Pyelonephritis

Chronic pyelonephritis is a chronic tubulointerstitial disease resulting from repeated attacks of inflammation and scarring.

ETIOPATHOGENESIS. Depending upon the etiology and pathogenesis, two types of chronic pyelonephritis are described—reflux nephropathy and obstructive pyelonephritis.

1. Reflux nephropathy. Reflux of urine from the bladder into one or both the ureters during micturition is the major cause of chronic pyelonephritis. *Vesicoureteric reflux* is particularly common in children, especially in girls, due to congenital absence or shortening of the intravesical portion of the ureter so that ureter is not compressed during the act of micturition. Reflux results in increase in pressure in the renal pelvis so that the urine is forced into renal tubules which is eventually followed by damage to the kidney and scar formation (Fig. 29.8). Vesicoureteric reflux is

more common in patients with urinary tract infection, whether symptomatic or asymptomatic, but reflux of sterile urine can also cause renal damage.

2. Obstructive pyelonephritis. Obstruction to the outflow of urine at different levels predisposes the kidney to infection. Recurrent episodes of such obstruction and infection result in renal damage and scarring. Rarely, recurrent attacks of acute pyelonephritis may cause renal damage and scarring.

The two major mechanisms of chronic pyelonephritis are diagrammatically illustrated in Fig. 29.9.

PATHOLOGIC CHANGES. *Grossly*, the kidneys show rather characteristic appearance. The kidneys are usually small and contracted (weighing less than 100 gm) showing unequal reduction so as to distinguish it from other forms of contracted kidney. The surface of the kidney is irregularly scarred; the capsule can be stripped off with difficulty due to adherence to scars. These scars are of variable size and show characteristic U-shaped depressions on the cortical surface. There is generally dilatation of pelvis and blunted calyces (Fig. 29.10).

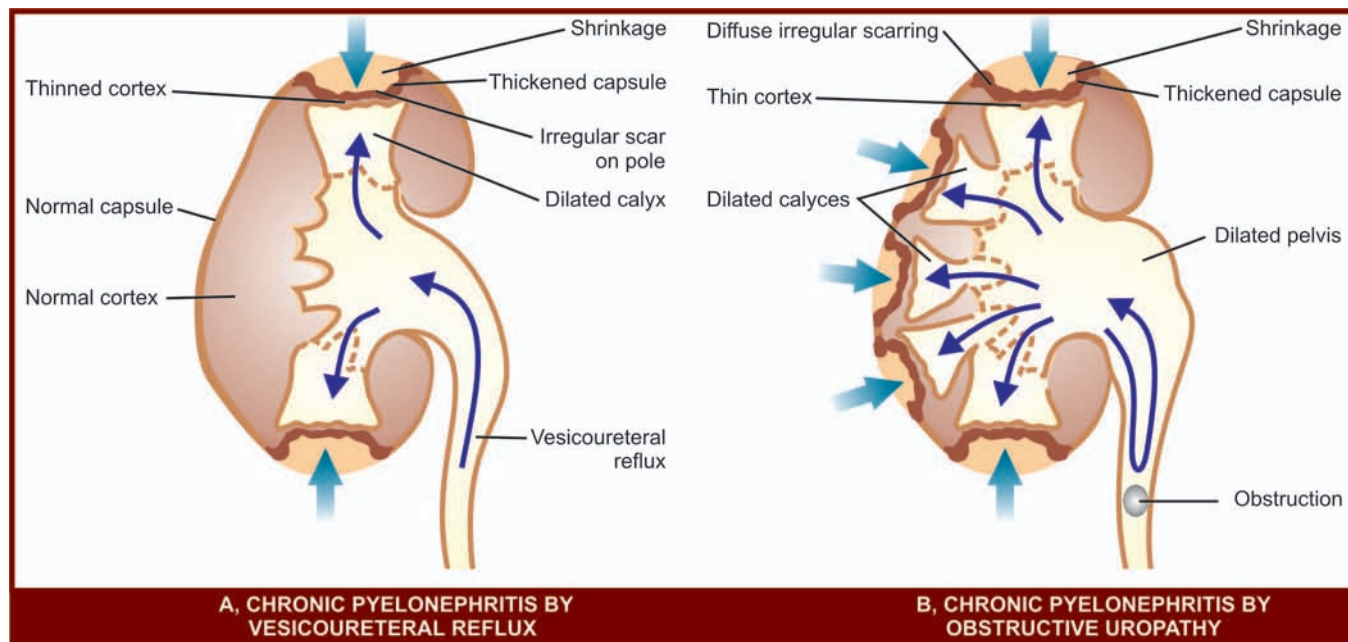


FIGURE 29.9

The two major types of chronic pyelonephritis. A, *Vesicoureteric reflux* causing infection of peripheral papillae and consequent scars at the poles of the kidney. B, *Obstructive pyelonephritis* due to obstruction of the urinary tract causing high pressure backflow of urine and infection of all the papillae and consequent diffuse scarring of the kidney and thinning of the cortex.

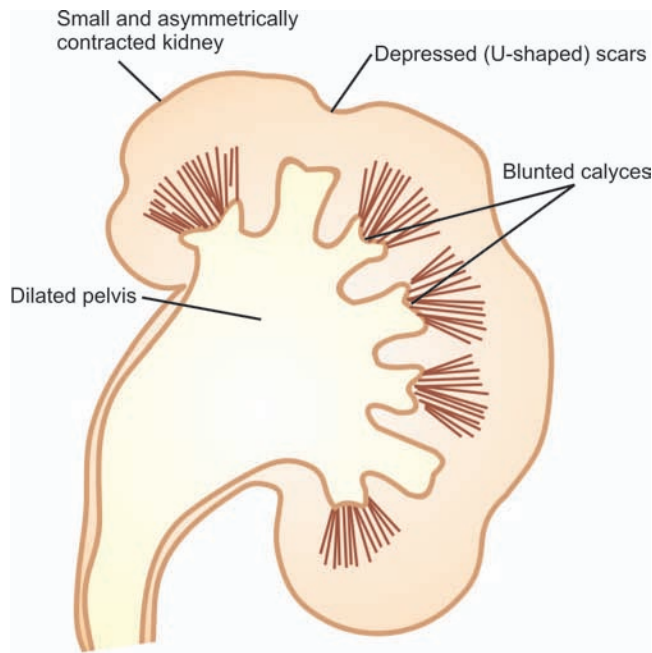


FIGURE 29.10

Chronic pyelonephritis, gross appearance. The kidney is asymmetrically small and contracted with characteristic U-shaped depressed scars on the cortical surface. There is dilatation of renal pelvis and blunted calyces.

Microscopically, predominant changes are seen in interstitium and tubules (Fig. 29.11):

i) Interstitium—There is chronic interstitial inflammatory reaction, chiefly composed of lymphocytes, plasma cells and macrophages with pronounced interstitial fibrosis. *Xanthogranulomatous pyelonephritis* is an uncommon variant characterised by collection of foamy macrophages admixed with other inflammatory cells and giant cells.

ii) Tubules—The tubules show varying degree of atrophy and dilatation. Dilated tubules may contain eosinophilic colloid casts producing thyroidisation of tubules. A few tubules may contain neutrophils.

iii) Pelvicalyceal system—The renal pelvis and calyces are dilated. The walls of pelvis and calyces show marked chronic inflammation and fibrosis. Lymphoid follicles with germinal centres may be present in the pelvicalyceal walls.

iv) Blood vessels—Blood vessels entrapped in the scarred areas show obliterative endarteritis. There may be changes of hypertensive hyaline arteriosclerosis.

v) Glomeruli—Though glomerular tuft in the scarred area is usually intact, there is often peri-

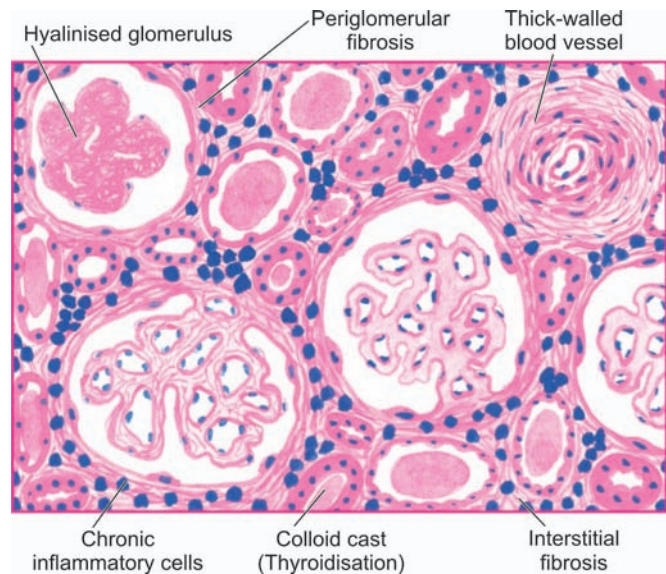


FIGURE 29.11

Chronic pyelonephritis, microscopic picture. The scarred area shows atrophy of some tubules and dilatation of others which contain colloid casts (thyroidisation). The tubules are surrounded by abundant fibrous tissue and chronic interstitial inflammatory reaction. The blood vessels included are thick-walled and the glomeruli show periglomerular fibrosis.

glomerular fibrosis. In advanced cases, there may be hyalinisation of glomeruli.

CLINICAL FEATURES. Chronic pyelonephritis often has an insidious onset. The patients present with clinical picture of chronic renal failure or with symptoms of hypertension. Sometimes, the patients may present with features of acute recurrent pyelonephritis with fever, loin pain, lumbar tenderness, dysuria, pyuria, bacteriuria and frequency of micturition. Diagnosis is made by intravenous pyelography (IVP). Culture of the urine may give positive results.

Tuberculous Pyelonephritis

Tuberculosis of the kidney occurs due to haematogenous spread of infection from another site, most often from the lungs. Less commonly, it may result from ascending infection from tuberculosis of the genitourinary system such as from epididymis or Fallopian tubes. The renal lesions in tuberculosis may be in the form of tuberculous pyelonephritis or appear as multiple miliary tubercles.

PATHOLOGIC CHANGES. *Grossly*, the lesions in tuberculous pyelonephritis are often bilateral, usually

involving the medulla with replacement of the papillae by caseous tissue. Obstruction may result in tuberculous pyonephrosis in which thinned out renal parenchyma surrounds dilated pelvis and calyces filled with caseous material.

Microscopically, typical granulomatous reaction is seen. Acid-fast bacilli can often be demonstrated in the lesions.

CLINICAL FEATURES. Most patients are young to middle-aged adults. The clinical presentation is extremely variable but it should always be considered as a possibility in a patient in whom there is persistent sterile pyuria, microscopic haematuria and mild proteinuria after effective antibiotic therapy for urinary tract infection. The diagnosis rests on identification of *M. tuberculosis* by repeated culture of urine on L.J. media.



30

Systemic Hypertension

Systemic hypertension i.e. an elevated arterial blood pressure, is a major health problem, particularly in the developed countries. Hypertension is associated with increased risk for a variety of cardiovascular disorders and is a major risk factor for atherosclerosis. Treatment of hypertension can prolong life. A persistent and sustained high blood pressure has damaging effects on heart (hypertensive heart disease), brain (stroke or cerebrovascular-accident), kidneys (benign and malignant nephrosclerosis), and eyes (hypertensive retinopathy).

DEFINITION AND CLASSIFICATION

Arterial or systemic hypertension in a patient is defined clinically as '*borderline*' if the systolic blood pressure is more than 140 mm Hg, and diastolic pressure is above 90 mm Hg (currently labelled as *mild hypertension*), and '*hypertensive*' when the elevation of systolic and diastolic pressure exceeds 160 and 95 mm Hg respectively (now termed as *moderate hypertension*). The diastolic pressure is often considered more significant. However, blood pressure varies with many factors such as age of the patient, exercise, emotional disturbances like fear and anxiety. Therefore, it is important to measure blood pressure at least twice during two separate examinations under least stressful conditions. A clinically useful classification of hypertension has been recently described by the Joint National Committee of the WHO/International Society of Hypertension (Table 30.1). By means of these criteria, the prevalence of hypertension is observed in about 25% of population.

Hypertension is generally classified into 2 types:

1. **Primary or essential hypertension** in which the cause of increase in blood pressure is unknown. Essential hypertension constitutes about 90-95% patients of hypertension.
2. **Secondary hypertension**, in which the increase in blood pressure is caused by diseases of the kidneys, endocrines or some other organs. Secondary hypertension comprises 5-10% cases of hypertension.

According to the clinical course, both essential and secondary hypertension may be benign or malignant.

TABLE 30.1: Clinical Classification of Hypertension.*

CATEGORY	SYSTOLIC (mm Hg)	DIASTOLIC (mm Hg)
<i>Normal</i>	< 130	< 85
<i>High normal</i>	130-139	85-89
<i>Hypertension</i>		
<i>mild (stage 1)</i>	140-159	90-99
<i>moderate (stage 2)</i>	160-179	100-109
<i>severe (stage 3)</i>	180-209	110-119
<i>very severe (stage 4)</i>	≥ 210	≥ 120
<i>Malignant hypertension</i>	> 200	≥ 140

*WHO/International Society of Hypertension (ISH) Special Report, 1995.

■ **Benign hypertension** is moderate elevation of blood pressure and the rise is slow over the years. About 90-95% patients of hypertension have benign hypertension.

■ **Malignant hypertension** is marked and rapid increase of blood pressure to 200/140 mm Hg or more and the patients have papilloedema, retinal haemorrhages and hypertensive encephalopathy. Less than 5% of hypertensive patients develop malignant hypertension and life expectancy after diagnosis in these patients is generally less than 2 years if not treated effectively.

ETIOLOGY AND PATHOGENESIS

The etiology and pathogenesis of secondary hypertension that comprises less than 10% cases has been better understood, whereas the mechanism of essential hypertension that constitutes about 90% of cases remains largely obscure. In general, normal blood pressure is regulated by 2 haemodynamic forces—*cardiac output* and *total peripheral vascular resistance*. Factors which alter these two factors result in hypertension. The role of kidney in hypertension, particularly in secondary hypertension, by elaboration of renin and subsequent formation of angiotensin II, is well established (renin-angiotensin system).

With this background knowledge, we next turn to the mechanisms involved in the two forms of hypertension (Table 30.2).

TABLE 30.2: Etiologic Classification of Hypertension.

A. ESSENTIAL HYPERTENSION (90%)

1. Genetic factors
2. Racial and environmental factors
3. Risk factors modifying the course

B. SECONDARY HYPERTENSION (10%)

1. Renal
 - i) Renovascular
 - ii) Renal parenchymal diseases
2. Endocrine
 - i) Adrenocortical hyperfunction
 - ii) Hyperparathyroidism
 - iii) Oral contraceptives
3. Coarctation of Aorta
4. Neurogenic

ESSENTIAL (PRIMARY) HYPERTENSION. By definition, the cause of essential hypertension is unknown but a number of factors are related to its development. These are as under:

1. Genetic factors. The role of heredity in the etiology of essential hypertension has long been suspected. The evidences in support are the familial aggregation, occurrence of hypertension in twins, epidemiologic data, experimental animal studies and identification of hypertension susceptibility gene (angiotensinogen gene).

2. Racial and environmental factors. Surveys in the US have revealed higher incidence of essential hypertension in blacks than in whites. A number of environmental factors have been implicated in the development of hypertension including salt intake, obesity, skilled occupation, higher living standards and patients in high stress.

3. Risk factors modifying the course of essential hypertension. There is sufficient evidence to show that the course of essential hypertension that begins in middle life is modified by a number of factors. These are as under:

- i) *Age.* Younger the age at which hypertension is first noted but left untreated, lower the life expectancy.
- ii) *Sex.* Females with hypertension appear to do better than males.
- iii) *Atherosclerosis.* Accelerated atherosclerosis invariably accompanies essential hypertension. This could be due to contributory role of other independent factors like cigarette smoking, elevated serum cholesterol, glucose intolerance and obesity.
- iv) *Other risk factors.* Other factors which alter the prognosis in hypertension include: smoking, excess of alcohol

intake, diabetes mellitus, persistently high diastolic pressure above 115 mm Hg and evidence of end-organ damage (i.e. heart, eyes, kidney and nervous system).

The *pathogenetic mechanism* in essential hypertension is explained by many theories. These are:

1. *High plasma level of catecholamines.*
2. *Increase in blood volume* i.e. arterial overfilling (volume hypertension) and arteriolar constriction (vasoconstrictor hypertension).
3. *Increased cardiac output.*
4. *Low-renin essential hypertension* found in approximately 20% patients due to altered responsiveness to renin release.
5. *High renin essential hypertension* seen in about 15% cases due to decreased adrenal responsiveness to angiotensin II.

SECONDARY HYPERTENSION. Mechanisms underlying hypertension with identifiable cause have been studied more extensively. Based on the etiology, these are described under four headings: renal hypertension, endocrine hypertension, hypertension associated with coarctation of aorta and neurogenic causes.

1. RENAL HYPERTENSION. Hypertension produced by renal diseases is called renal hypertension. Renal hypertension is subdivided into 2 groups:

- i) *Renal vascular hypertension* e.g. in occlusion of a major renal artery, pre-eclampsia, eclampsia, polyarteritis nodosa and fibromuscular dysplasia of renal artery.
- ii) *Renal parenchymal hypertension* e.g. in various types of glomerulonephritis, pyelonephritis, interstitial nephritis, diabetic nephropathy, amyloidosis, polycystic kidney disease and renin-producing tumours.

In either case, renal hypertension can be produced by one of the 3 inter-related pathogenetic mechanisms:

- activation of renin-angiotensin system,
- sodium and water retention, and
- decreased release of vasodepressor materials.

a) Activation of renin-angiotensin system. Renin is a proteolytic enzyme produced and stored in the granules of the juxtaglomerular cells surrounding the afferent arterioles of glomerulus. The release of renin is stimulated by renal ischaemia, sympathetic nervous system stimulation, depressed sodium concentration, fluid depletion and decreased potassium intake. Released renin is transported through blood stream to the liver where it acts upon substrate angiotensinogen, an α_2 -globulin synthesised in the liver, to form angiotensin I, a decapeptide. Angiotensin I is converted into angiotensin II, an octapeptide, by the action of

convertase in the lungs. Angiotensin II is the most potent naturally-occurring vasoconstrictor substance and its pressor action is mainly attributed to peripheral arteriolar vasoconstriction. The other main effect of angiotensin II is to stimulate the adrenal cortex to secrete aldosterone that promotes reabsorption of sodium and water.

Thus, the renin-angiotensin system is concerned mainly with 3 functions:

- i) Control of blood pressure by altering plasma concentration of angiotensin II and aldosterone.
- ii) Regulation of sodium and water content.
- iii) Regulation of potassium balance.

The renin-angiotensin mechanism is summarised in Fig. 30.1.

b) Sodium and water retention. Blood volume and cardiac output, both of which have a bearing on blood pressure, are regulated by blood levels of sodium which is significant for maintaining extracellular fluid volume. Blood concentration of sodium is regulated by 3 mechanisms:

- i) *Release of aldosterone* from activation of renin-angiotensin system, as already explained.
- ii) *Reduction in GFR* due to reduced blood flow as occurs in reduced renal mass or renal artery stenosis. This results in proximal tubular reabsorption of sodium.
- iii) *Release of atriopeptin hormone* from atria of the heart in response to volume expansion. These peptides cause increased GFR and inhibit sodium reabsorption.

c) Release of vasodepressor material. A number of vasodepressor materials and antihypertensives counterbalance the vasopressor effect of angiotensin II. These substances include: prostaglandins (PGE₂, PGF₂, PGA or medullin) released from interstitial cells of the medulla, urinary kallikrein-kinin system and platelet-activating factor.

2. ENDOCRINE HYPERTENSION. A number of hormonal secretions may produce secondary hypertension. These are:

- i) *Adrenal gland*—e.g. in primary aldosteronism, Cushing's syndrome, adrenal virilism and pheochromocytoma.
- ii) *Parathyroid gland*—e.g. hypercalcaemia in hyperparathyroidism.
- iii) *Oral contraceptives*—Oestrogen component in the oral contraceptives stimulates hepatic synthesis of renin substrate.

3. COARCTATION OF AORTA. Coarctation of the aorta causes systolic hypertension in the upper part of the body due to constriction itself. Diastolic hypertension results from changes in circulation.

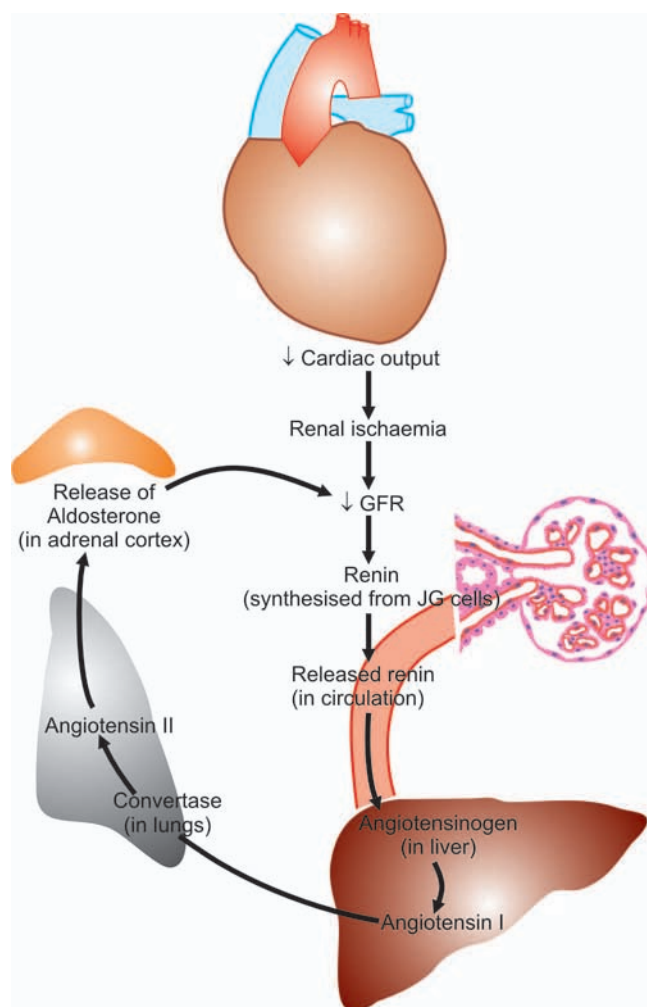


FIGURE 30.1

The renin-angiotensin mechanism.

4. NEUROGENIC. Psychogenic, polyneuritis, increased intracranial pressure and section of spinal cord are all uncommon causes of secondary hypertension.

EFFECTS OF HYPERTENSION

Systemic hypertension causes major effects in the following main organs—

1. kidneys (benign and malignant nephrosclerosis);
2. heart and its blood vessels (hypertensive heart disease);
3. eyes (hypertensive retinopathy); and
4. nervous system (intracranial haemorrhage).

1. NEPHROSCLEROSIS

The renal effects of hypertension are in the form of benign and malignant nephrosclerosis.

Benign Nephrosclerosis

Benign nephrosclerosis is the term used to describe the kidney of benign phase of hypertension. Mild benign nephrosclerosis is the most common form of renal disease in persons over 60 years of age but its severity increases in the presence of hypertension and diabetes mellitus.

PATHOLOGIC CHANGES. *Grossly*, both the kidneys are affected equally and are reduced in size and weight, often weighing about 100 gm or less. The capsule is often adherent to the cortical surface. The surface of the kidney is finely granular and shows V-shaped areas of scarring.* The cut surface shows firm kidney and narrowed cortex (Fig. 30.2,A).

Microscopically, there are primarily diffuse vascular changes which produce parenchymal changes secondarily as a result of ischaemia. The histologic changes are, thus, described as vascular and parenchymal (Fig. 30.2,B):

i) Vascular changes: Changes in blood vessels involve arterioles and arteries up to the size of arcuate arteries. There are 2 types of changes in these blood vessels:

a) *Hyaline arteriosclerosis* that results in homogeneous and eosinophilic thickening of the wall of small blood vessels.

b) *Intimal thickening* due to proliferation of smooth muscle cells in the intima.

ii) Parenchymal changes: As a consequence of ischaemia, there is variable degree of atrophy of parenchyma. This includes: glomerular shrinkage, deposition of collagen in Bowman's space, periglomerular fibrosis, tubular atrophy and fine interstitial fibrosis.

CLINICAL FEATURES. There is variable elevation of the blood pressure with headache, dizziness, palpitation and nervousness. Eye ground changes may be found but papilloedema is absent. Renal function tests and urine examination are normal in early stage. But in long-

*The various acquired causes of 'small contracted kidney' and their characteristic gross macroscopic appearance may be recollected here. These are: 1. Chronic GN (granular appearance); 2. Chronic pyelonephritis (U-shaped scars); and 3. Benign nephrosclerosis (V-shaped scars). Although granular, U- and V-shaped scars correspond to the respective macroscopic patterns, an easy way to remember is: 'granular' for glomerular scars of chronic GN; 'U-scars' for uneven scars of chronic pyelonephritis; and 'V-scars' for vascular scars of benign nephrosclerosis. Less common causes are: amyloidosis of the kidney, myeloma kidney and diabetic nephropathy.

standing cases, there may be mild proteinuria with some hyaline or granular casts. Rarely, renal failure and uraemia may occur.

Malignant Nephrosclerosis

Malignant nephrosclerosis is the form of renal disease that occurs in malignant or accelerated hypertension. Malignant nephrosclerosis is uncommon and usually occurs as a superimposed complication in 5% cases of pre-existing benign essential hypertension or in those having secondary hypertension with identifiable cause such as in chronic renal diseases. However, the pure form of disease also occurs, particularly at younger age with preponderance in males.

PATHOLOGIC CHANGES. *Grossly*, the appearance of the kidney varies. In a case of malignant hypertension superimposed on pre-existing benign nephrosclerosis, the kidneys are small in size, shrunken and reduced in weight and have finely granular surface. However, the kidneys of a patient who develops malignant hypertension in pure form are enlarged, oedematous and have petechial haemorrhages on the surface producing so called 'flea-bitten kidney'.* Cut surface shows red and yellow mottled appearance (Fig. 30.3,A).

Microscopically, most commonly the changes are superimposed on benign nephrosclerosis. These changes are as under (Fig. 30.3,B):

i) Vascular changes: These are more severe and involve the arterioles. The two characteristic vascular changes seen are as under:

a) *Necrotising arteriolitis* develops on hyaline arteriosclerosis. The vessel wall shows fibrinoid necrosis, a few acute inflammatory cells and small haemorrhages.

b) *Hyperplastic intimal sclerosis* or *onionskin proliferation* is characterised by concentric laminae of proliferated smooth muscle cells, collagen and basement membranes.

ii) Ischaemic changes: The effects of vascular narrowing on the parenchyma include tubular loss, fine interstitial fibrosis and foci of infarction necrosis.

CLINICAL FEATURES. The patients of malignant nephrosclerosis have malignant or accelerated hypertension with blood pressure of 200/140 mm Hg or

*Recall the other causes of *flea-bitten kidney*: acute post-streptococcal GN, rapidly progressive GN, haemolytic-uraemic syndrome, thrombotic thrombocytopenic purpura and Hensch-Schonlein purpura.

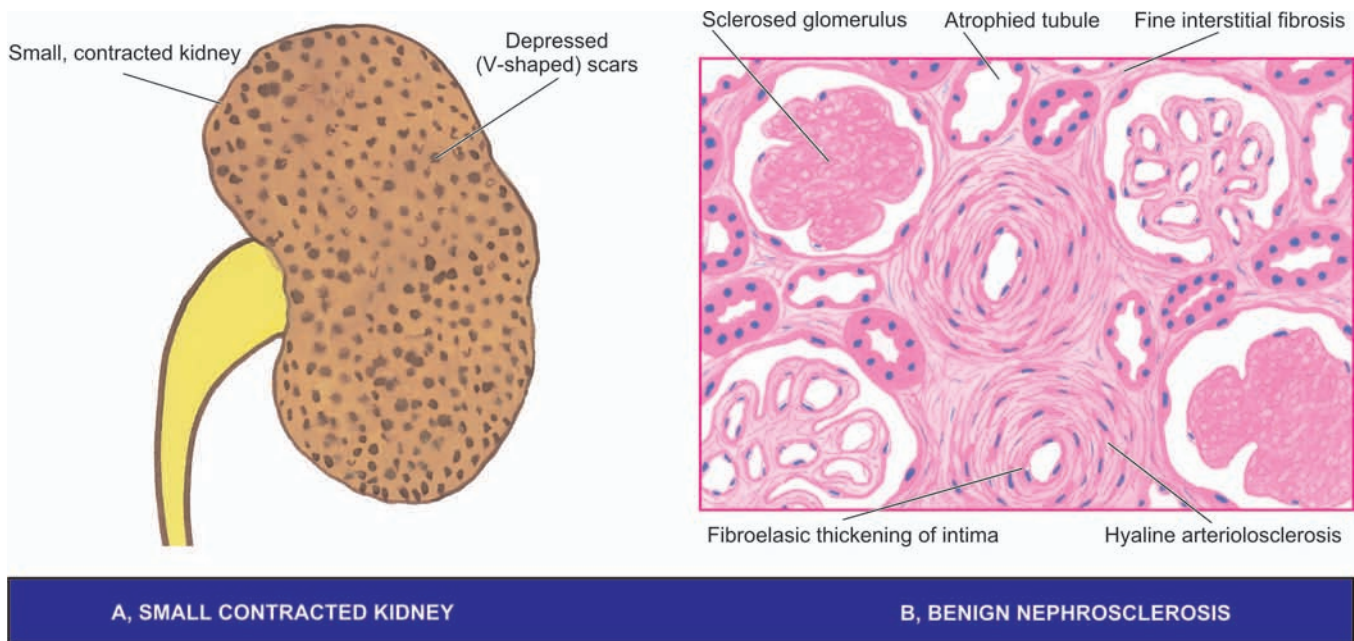


FIGURE 30.2

Benign nephrosclerosis. A, Gross appearance, showing characteristic 'small contracted kidney' with depressed V-shaped scars. B, Microscopic appearance. The vascular changes are hyaline arteriosclerosis and intimal thickening of small blood vessels in the glomerular tuft. The parenchymal changes include sclerosed glomeruli, tubular atrophy and fine interstitial fibrosis.

higher. Headache, dizziness and impaired vision are commonly found. The presence of papilloedema distinguishes malignant from benign phase of hypertension. The urine frequently shows haematuria and proteinuria. Renal function tests show deterioration during the course of the illness. Azotaemia (high BUN and serum creatinine) and uraemia develop soon if malignant hypertension is not treated aggressively. Approximately 90% of patients die within one year from causes such as uraemia, congestive heart failure and cerebrovascular accidents.

2. HYPERTENSIVE HEART DISEASE

Hypertensive heart disease or hypertensive cardiomyopathy is the disease of the heart resulting from systemic hypertension of prolonged duration and manifesting by left ventricular hypertrophy. Even mild hypertension (blood pressure higher than 140/90 mm Hg) of sufficient duration may induce hypertensive heart disease. It is the second most common form of heart disease after IHD. As already pointed out, hypertension predisposes to atherosclerosis. Therefore, most patients of hypertensive heart disease have advanced coronary atherosclerosis and may develop progressive IHD. Amongst the causes of death in hypertensive

patients, cardiac decompensation leading to CHF accounts for about one-third of the patients; other causes of death are IHD, cerebrovascular stroke, renal failure following arteriolar nephrosclerosis, and dissecting aneurysm of the aorta.

PATHOGENESIS. Pathogenesis of left ventricular hypertrophy which is most commonly caused by systemic hypertension is described here.

Stimulus to hypertrophy of the left ventricle is pressure overload in systemic hypertension. The stress of pressure on the ventricular wall causes increased production of myofilaments, myofibrils, other cell organelles and nuclear enlargement. Since the adult myocardial fibres do not divide, the fibres are hypertrophied. However, the sarcomeres may divide to increase the cell width.

PATHOLOGIC CHANGES. *Grossly*, the most significant finding is marked hypertrophy of the heart, chiefly of the left ventricle (Fig. 30.4). The weight of the heart increases to 500 gm or more (normal weight about 300 gm). The thickness of the left ventricular wall increases from its normal 13 to 15 mm upto 20 mm or more. The papillary muscles and trabeculae carneae are rounded and prominent. Initially, there is *concentric hypertrophy* of the left ventricle (without

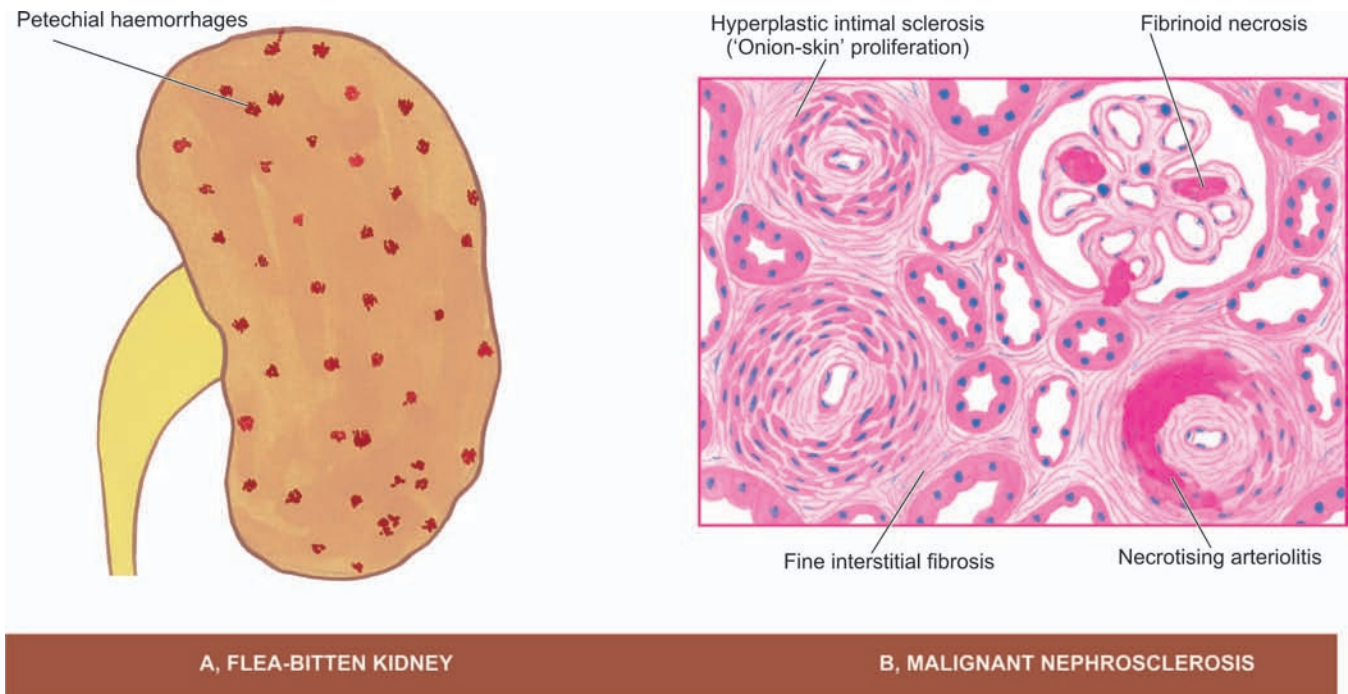


FIGURE 30.3

Malignant nephrosclerosis. A, Gross appearance, showing characteristic 'flea bitten kidney' due to tiny petechial haemorrhages on the surface. B, Microscopic appearance. The vascular changes are necrotising arteriolitis and hyperplastic intimal sclerosis or onionskin proliferation. The parenchymal changes are tubular loss, fine interstitial fibrosis and foci of infarction necrosis.

dilatation). But when decompensation and cardiac failure supervene, there is *eccentric hypertrophy* (with dilatation) with thinning of the ventricular wall and there may be dilatation and hypertrophy of right heart as well.

Microscopically, the features are not as prominent as macroscopic appearance. The changes include enlargement and degeneration of myocardial fibres with focal areas of myocardial fibrosis. In advanced cases, there may be myocardial oedema and foci of necrosis in the myocardium.

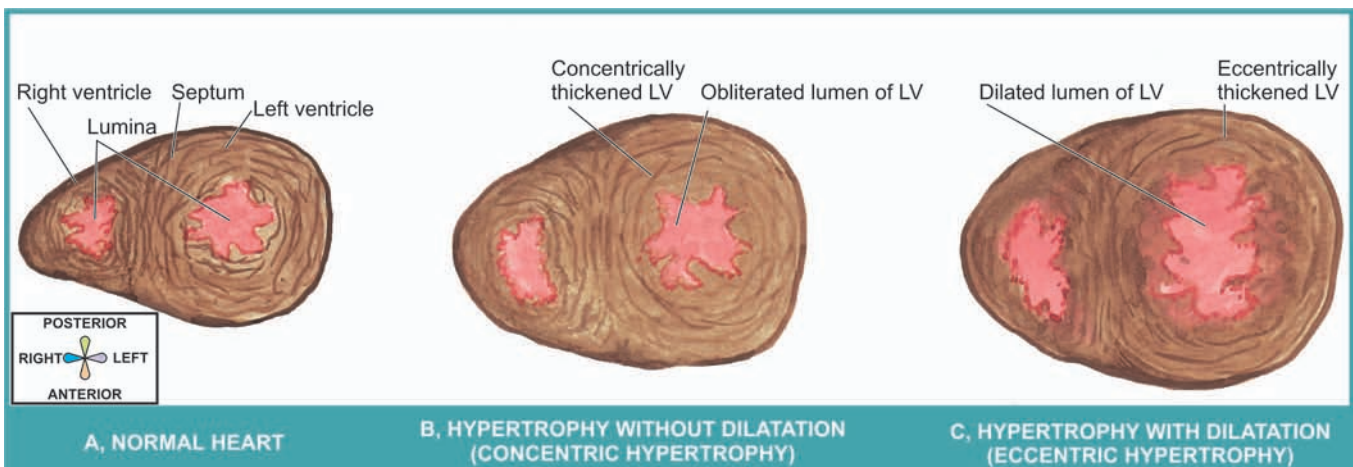


FIGURE 30.4

Transverse section through the ventricles showing left ventricular hypertrophy.

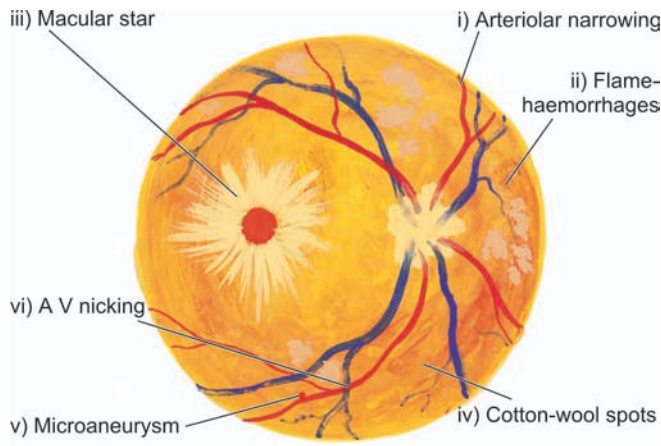


FIGURE 30.5

Ocular lesions in hypertension.

3. HYPERTENSIVE RETINOPATHY

In hypertensive retinopathy, the retinal arterioles are reduced in their diameter leading to retinal ischaemia. In acute severe hypertension as happens at the onset of malignant hypertension and in toxæmia of pregnancy, the vascular changes are in the form of spasms, while in chronic hypertension the changes are diffuse in the form of onion-skin thickening of the arteriolar walls with narrowing of the lumina.

Features of hypertensive retinopathy include the following (Fig. 30.5):

- i) Variable degree of arteriolar narrowing due to arteriosclerosis.
- ii) 'Flame-shaped' haemorrhages in the retinal nerve fibre layer.
- iii) Macular star i.e. exudates radiating from the centre of macula.
- iv) Cotton-wool spots i.e. fluffy white bodies in the superficial layer of retina.
- v) Microaneurysms.
- vi) Arteriovenous nicking i.e. kinking of veins at sites where sclerotic arterioles cross veins.
- vii) Hard exudates due to leakage of lipid and fluid into macula.

Hypertensive retinopathy is classified according to the severity of above lesions from grade I to IV. More serious and severe changes with poor prognosis occur in higher grades of hypertensive retinopathy. Malignant hypertension is characterised by necrotising arteriolitis and fibrinoid necrosis of retinal arterioles.

4. INTRACRANIAL HAEMORRHAGE

Haemorrhage into the brain may be traumatic, non-traumatic, or spontaneous. There are two main types of spontaneous intracranial haemorrhage:

1. Intracerebral haemorrhage, which is usually of hypertensive origin.
2. Subarachnoid haemorrhage, which is commonly aneurysmal in origin.

In addition to hypertension and rupture of an aneurysm, other causes of spontaneous intracranial haemorrhage include vascular malformations which produce mixed intracerebral and subarachnoid haemorrhage, haemorrhagic diathesis and haemorrhage into tumours.

Intracerebral Haemorrhage

Spontaneous intracerebral haemorrhage occurs mostly in patients of hypertension. Most hypertensives over middle age have microaneurysms in very small cerebral arteries in the brain tissue. Rupture of one of the numerous microaneurysms is believed to be the cause of intracerebral haemorrhage. Unlike subarachnoid haemorrhage, it is not common to have recurrent intracerebral haemorrhages.

The common sites of hypertensive intracerebral haemorrhage are the region of the basal ganglia (particularly the putamen and the internal capsule), pons and the cerebellar cortex. Clinically the onset is usually sudden with headache and loss of consciousness. Depending upon the location of the lesion, hemispheric, brainstem or cerebellar signs will be present. About 40% of patients die during the first 3-4 days of haemorrhage, mostly from haemorrhage into the ventricles. The survivors tend to have haematoma that separates the tissue planes which is followed by resolution and development of an *apoplectic cyst* accompanied by loss of function.

PATHOLOGIC CHANGES. *Grossly* and *microscopically*, the haemorrhage consists of dark mass of clotted blood replacing brain parenchyma. The borders of the lesion are sharply-defined and have a narrow rim of partially necrotic parenchyma. Small ring haemorrhages in the Virchow-Robin space in the border zone are commonly present. Ipsilateral ventricles are distorted and compressed and may contain blood in their lumina. Rarely, blood may rupture through the surface of the brain into the subarachnoid space. After a few weeks to months, the haematoma undergoes resolution with formation of a slit-like space called *apoplectic cyst* and containing yellowish fluid. Its margins are yellow-brown and

contain haemosiderin-laden macrophages and a reactive zone of fibrillary astrocytosis.

Subarachnoid Haemorrhage

Haemorrhage into the subarachnoid space is most commonly caused by rupture of an aneurysm, and rarely, rupture of a vascular malformation.

Of the three types of aneurysms affecting the larger intracranial arteries, namely berry, mycotic and fusiform, **berry aneurysms** are most important and most common. Berry aneurysms are saccular in appearance with rounded or lobulated bulge arising at the bifurcation of intracranial arteries and varying in size from 2 mm to 2 cm or more. They account for 95% of aneurysms which are liable to rupture. Berry aneurysms are rare in childhood but increase in frequency in young adults and middle life. They are, therefore, not congenital anomalies but develop over the years from developmental defect of the media of the arterial wall at the bifurcation of arteries forming thin-walled saccular bulges. Although most berry aneurysms are sporadic in occurrence, there is an increased incidence of their presence in association with congenital polycystic kidney disease and coarctation of the aorta. About a quarter of berry aneurysms are multiple.

In more than 85% cases of subarachnoid haemorrhage, the cause is massive and sudden bleeding from a berry aneurysm on or near the circle of Willis. The four most common sites are (Fig. 30.6):

1. in relation to anterior communicating artery;
2. at the origin of the posterior communicating artery from the stem of the internal carotid artery;
3. at the first major bifurcation of the middle cerebral artery;
4. at the bifurcation of the internal carotid into the middle and anterior cerebral arteries.

The remaining 15% cases of subarachnoid haemorrhage are the result of rupture in the posterior circulation, vascular malformations and rupture of mycotic aneurysms that occurs in the setting of bacterial endocarditis. In all types of aneurysms, the rupture of thin-walled dilatation occurs in association with sudden rise

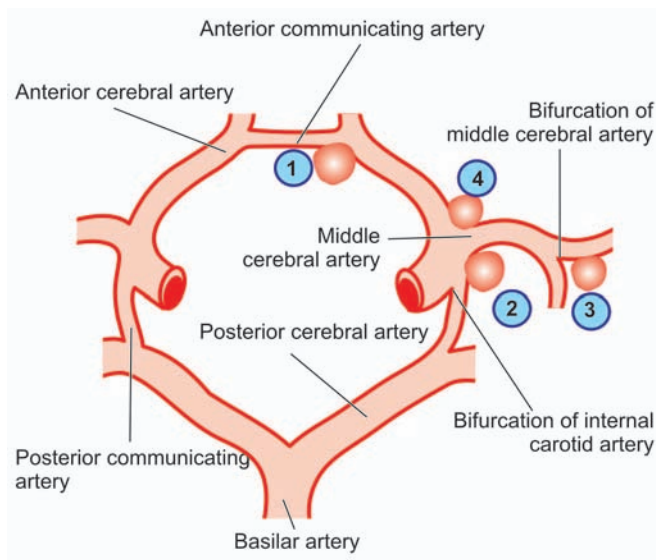


FIGURE 30.6

The circle of Willis showing principal sites of berry (saccular) aneurysms. The serial numbers indicate the frequency of involvement.

in intravascular pressure but chronic hypertension does not appear to be a risk factor in their development or rupture.

Clinically, berry aneurysms remain asymptomatic prior to rupture. On rupture, they produce severe generalised headache of sudden onset which is frequently followed by unconsciousness and neurologic defects. Initial mortality from first rupture is about 20-25%. Survivors recover completely but frequently suffer from recurrent episodes of fresh bleeding.

PATHOLOGIC CHANGES. Rupture of a berry aneurysm frequently spreads haemorrhage throughout the subarachnoid space with rise in intracranial pressure and characteristic blood-stained CSF. An intracerebral haematoma may develop if the blood tracks into the brain parenchyma. The region of the brain supplied by the affected artery frequently shows infarction, partly attributed to vasospasm.



Diabetes Mellitus

DEFINITION AND EPIDEMIOLOGY

As per the WHO, diabetes mellitus (DM) is a heterogeneous metabolic disorder characterised by common feature of chronic hyperglycaemia with disturbance of carbohydrate, fat and protein metabolism.

DM is a leading cause of morbidity and mortality the world over. It is estimated that approximately 1% of population suffers from DM. The incidence is rising in the developed countries of the world, especially of type 2 DM, due to rising incidence of obesity and reduced activity levels. DM is expected to continue as a major health problem owing to its serious complications, especially end-stage renal disease, IHD, gangrene of the lower extremities and blindness in the adults.

CLASSIFICATION AND ETIOLOGY

The older classification systems dividing DM into primary (idiopathic) and secondary types, juvenile-onset and maturity onset types, and insulin-dependent (IDDM) and non-insulin dependent (NIDDM) types, have become obsolete and undergone major revision due to extensive understanding of etiology and pathogenesis of DM in recent times.

As outlined in Table 31.1 based on etiology, currently DM is classified into two broad categories (type 1 and type 2), a few uncommon specific etiologic types, and gestational DM. Brief comments on these etiologic terminologies as contrasted with former nomenclatures of DM are given below:

TYPE 1 DM. It constitutes about 10% cases of DM. It was previously termed as juvenile-onset diabetes (JOD) due to its occurrence in younger age, and was called insulin-dependent DM (IDDM) because these patients had absolute requirement for insulin replacement as treatment. However, in the new classification, neither age nor insulin-dependence are considered as absolute criteria. Instead, based on underlying etiology, type 1 DM is further divided into 2 subtypes:

Subtype 1A (immune-mediated) DM characterised by autoimmune destruction of β -cells which usually leads to insulin deficiency.

TABLE 31.1: Etiologic Classification of Diabetes Mellitus (as per American Diabetes Association, 2000).

- I. **Type 1 diabetes mellitus (10%)** (earlier called Insulin-dependent, or juvenile-onset diabetes)
 - Type 1A DM:* Immune-mediated
 - Type 1B DM:* Idiopathic
- II. **Type 2 diabetes mellitus (80%)** (earlier called non-insulin-dependent, or maturity-onset diabetes)
- III. **Other specific types of diabetes (10%)**
 - A. Genetic defect of β -cell function due to mutations in various enzymes (earlier called maturity-onset diabetes of the young or MODY) (e.g. hepatocyte nuclear transcription factor HNF, glucokinase)
 - B. Genetic defect in insulin action (e.g. type A insulin resistance)
 - C. Diseases of exocrine pancreas (e.g. chronic pancreatitis, pancreatic tumours, post-pancreatectomy)
 - D. Endocrinopathies (e.g. acromegaly, Cushing's syndrome, pheochromocytoma)
 - E. Drug- or chemical-induced (e.g. steroids, thyroid hormone, thiazides, β -blockers etc)
 - F. Infections (e.g. congenital rubella, cytomegalovirus)
 - G. Uncommon forms of immune-mediated DM (stiff man syndrome, anti-insulin receptor antibodies)
 - H. Other genetic syndromes (e.g. Down's syndrome, Klinefelter's syndrome, Turner's syndrome)
- IV. **Gestational diabetes mellitus**

Subtype 1B (idiopathic) DM is characterised by insulin deficiency with tendency to develop ketosis but these patients are negative for autoimmune markers.

Though type 1 DM occurs commonly in patients under 30 years of age, autoimmune destruction of β -cells can occur at any age. In fact, 5-10% patients who develop DM above 30 years of age are of type 1A DM and hence the term JOD has become obsolete.

TYPE 2 DM. This type comprises about 80% cases of DM. It was previously called maturity-onset diabetes, or non-insulin dependent diabetes mellitus (NIDDM) of obese and non-obese type.

Although type 2 DM predominantly affects older individuals, it is now known that it also occurs in obese adolescent children; hence the term MOD for it is inappropriate. Moreover, many type 2 DM patients also require insulin therapy to control hyperglycaemia or to

prevent ketosis and thus are not truly non-insulin dependent contrary to its former nomenclature.

The contrasting features of the two main forms of diabetes mellitus are presented in Table 31.2.

OTHER SPECIFIC ETIOLOGIC TYPES OF DM.

Besides the two main types, about 10% cases of DM have a known specific etiologic defect; these are listed in Table 31.1. One important subtype in this group is *maturity-onset diabetes of the young (MODY)* which has autosomal dominant inheritance, early onset of hyperglycaemia and impaired insulin secretion.

GESTATIONAL DM. About 4% pregnant women develop DM due to metabolic changes during pregnancy. Although they revert back to normal glycaemia after delivery, these women are prone to develop DM later in their life.

PATHOGENESIS

Depending upon etiology of DM, hyperglycaemia may result from:

- reduced insulin secretion;
- decreased glucose use by the body; and
- increased glucose production.

Pathogenesis of two main types of DM and its complications is distinct. In order to understand it properly, it is essential to relearn physiology of normal insulin synthesis and secretion.

NORMAL INSULIN METABOLISM. *The major stimulus for both synthesis and release of insulin is glucose.*

The steps involved in biosynthesis, release and actions of insulin are as follows:

Synthesis. Insulin is synthesised in the β -cells of pancreatic islets of Langerhans (Fig. 31.1, A):

- i) It is initially formed as *pre-proinsulin* which is single-chain 86-amino acid precursor polypeptide.
- ii) Subsequent proteolysis removes the amino terminal signal peptide, forming *proinsulin*.
- iii) Further cleavage of proinsulin gives rise to *A* (21 amino acids) and *B* (30 amino acids) chains of insulin, linked together by connecting segment called *C-peptide*, all of which are stored in the secretory granules in the β -cells. As compared to A and B chains of insulin, C-peptide is less susceptible to degradation in the liver and is therefore used as a marker to distinguish endogenously synthesised and exogenously administered insulin.
- iv) For therapeutic use, *human insulin* is now produced by recombinant DNA technology.

Release. Glucose is the key regulator of insulin secretion from β -cells by a series of steps (Fig. 31.1,A):

- i) Hypoglycaemia (glucose level below 70 mg/dL or below 3.9 mmol/L) stimulates transport into β -cells of a *glucose transporter*, *GLUT2*. Other stimuli influencing insulin release include nutrients in the meal, ketones, amino acids etc.
- ii) An islet transcription factor, *glucokinase*, causes glucose phosphorylation, and thus acts as a step for controlled release of glucose-regulated insulin secretion.
- iii) Metabolism of glucose to glucose-6-phosphate by glycolysis generates *ATP*.

TABLE 31.2: Contrasting Features of Type 1 and Type 2 Diabetes Mellitus.

FEATURE	TYPE 1 DM	TYPE 2 DM
1. <i>Frequency</i>	10-20%	80-90%
2. <i>Age at onset</i>	Early (below 35 years)	Late (after 40 years)
3. <i>Type of onset</i>	Abrupt and severe	Gradual and insidious
4. <i>Weight</i>	Normal	Obese/non-obese
5. <i>HLA</i>	Linked to HLA DR3, HLA DR4, HLA DQ	No HLA association
6. <i>Family history</i>	< 20%	About 60%
7. <i>Genetic locus</i>	Unknown	Chromosome 6
8. <i>Diabetes in identical twins</i>	50% concordance	80% concordance
9. <i>Pathogenesis</i>	Autoimmune destruction of β -cells	Insulin resistance, impaired insulin secretion
10. <i>Islet cell antibodies</i>	Yes	No
11. <i>Blood insulin level</i>	Decreased insulin	Normal or increased insulin
12. <i>Islet cell changes</i>	Insulinitis, β -cell depletion	No insulinitis, later fibrosis of islets
13. <i>Amyloidosis</i>	Infrequent	Common in chronic cases
14. <i>Clinical management</i>	Insulin and diet	Diet, exercise, oral drugs, insulin
15. <i>Acute complications</i>	Ketoacidosis	Hyperosmolar coma

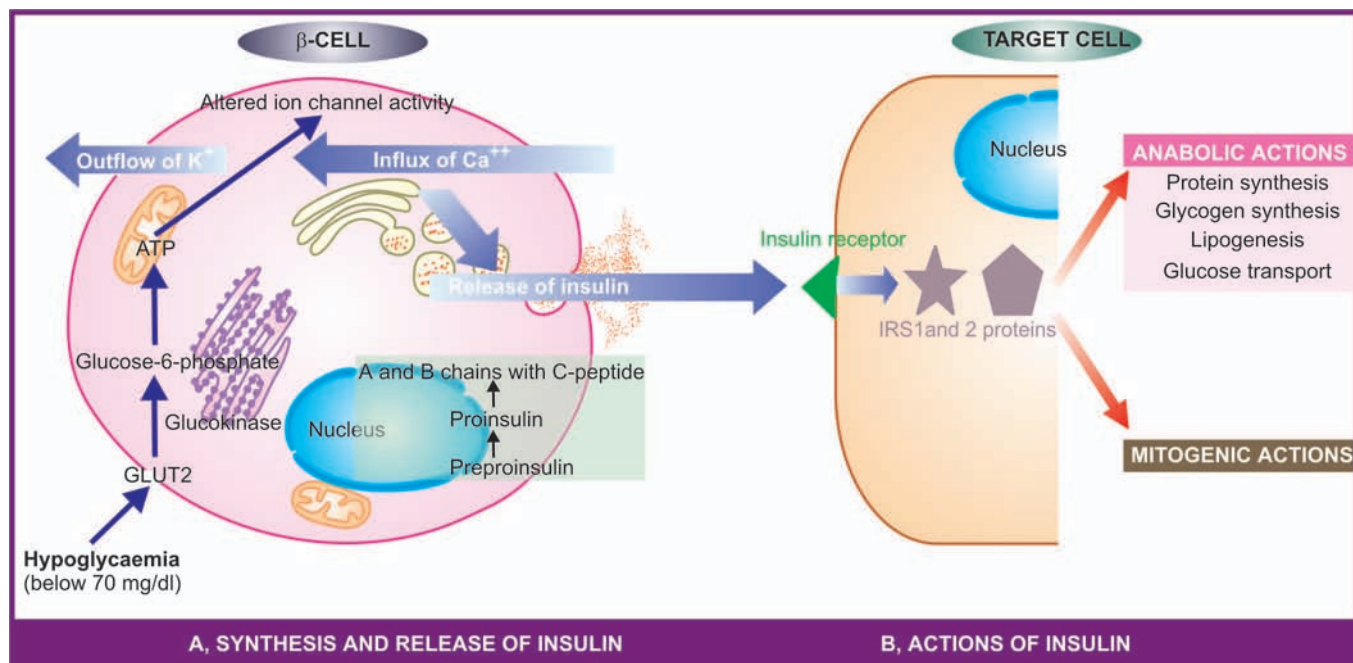


FIGURE 31.1

A, Pathway of normal insulin synthesis and release in β -cells of pancreatic islets. B, Chain of events in action of insulin on target cell.

iv) Generation of ATP alters the ion channel activity on the membrane. It causes inhibition of ATP-sensitive K^+ channel on the cell membrane and opening up of calcium channel with resultant influx of calcium, which stimulates insulin release.

Action. Half of insulin secreted from β -cells into portal vein is degraded in the liver while the remaining half enters the systemic circulation for action on the target cells (Fig. 31.1,B):

- i) Insulin from circulation binds to its receptor on the target cells. This *insulin receptor* has intrinsic tyrosine kinase activity.
- ii) This, in turn, activates post-receptor intracellular signalling pathway molecules, *insulin receptor substrates (IRS) 1 and 2 proteins*, which initiate sequence of phosphorylation and dephosphorylation reactions.
- iii) These reactions on the target cells are responsible for the main *mitogenic and anabolic actions of insulin*—glycogen synthesis, glucose transport, protein synthesis, lipogenesis.
- iv) Besides the role of glucose in maintaining equilibrium of insulin release, *low insulin level in the fasting state* promotes hepatic gluconeogenesis and glycogenolysis, reduced glucose uptake by insulin-sensitive tissues and promotes mobilisation of stored precursors, so as to prevent hypoglycaemia.

PATHOGENESIS OF TYPE 1 DM. The basic phenomena in type 1 DM is destruction of β -cell mass, usually leading to absolute insulin deficiency. While type 1B DM remains idiopathic, pathogenesis of type 1A DM is immune-mediated and has been extensively studied. Currently, pathogenesis of type 1A DM is explained on the basis of 3 mutually-interlinked mechanisms: genetic susceptibility, autoimmune factors, and certain environmental factors (Fig. 31.2,A).

1. Genetic susceptibility. Type 1A DM involves inheritance of multiple genes to confer susceptibility to the disorder:

- i) It has been observed in *identical twins* that if one twin has type 1A DM, there is about 50% chance of the second twin developing it, but not all. This means that some additional modifying factors are involved in development of DM in these cases.
- ii) About half the cases with genetic predisposition to type 1A DM have the *susceptibility gene* located in the HLA region of chromosome 6 (MHC class II region), particularly HLA DR3, HLA DR4 and HLA DQ locus.

It appears that in a HLA-associated susceptible individual, β -cells act as autoantigens and activate CD4+ T lymphocytes, bringing about immune destruction of pancreatic β -cells.

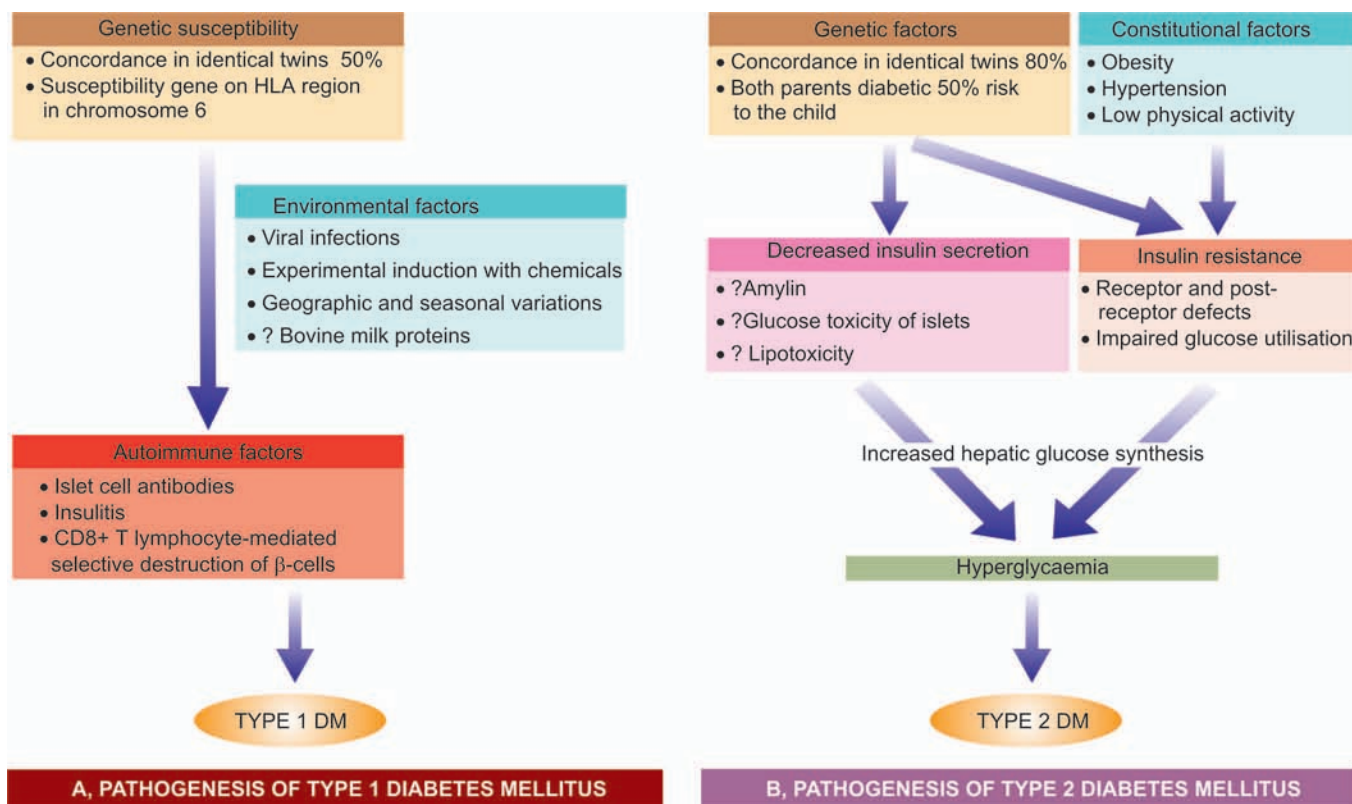


FIGURE 31.2

Schematic mechanisms involved in pathogenesis of two main types of diabetes mellitus.

2. Autoimmune factors. Studies on humans and animal models on type 1A DM have shown several immunologic abnormalities:

- Presence of *islet cell antibodies* against GAD (glutamic acid decarboxylase), insulin etc, though its assay largely remained as a research tool due to tedious method.
- Occurrence of lymphocytic infiltrate in and around the pancreatic islets termed *insulinitis*. It chiefly consists of CD8+ T lymphocytes with variable number of CD4+ T lymphocytes and macrophages.
- Selective destruction of β -cells* while other islet cell types (glucagon-producing alpha cells, somatostatin-producing delta cells, or polypeptide-forming PP cells) remain unaffected. This is mediated by T-cell mediated cytotoxicity or by apoptosis.
- Role of *T cell-mediated autoimmunity* is further supported by transfer of type 1A DM from diseased animal by infusing T lymphocytes to a healthy animal.
- Association of type 1A DM with *other autoimmune diseases* in about 10-20% cases such as Graves' disease, Addison's disease, Hashimoto's thyroiditis, pernicious anaemia.

vi) Remission of type 1A DM in response to immuno-suppressive therapy such as administration of cyclosporin A.

3. Environmental factors. Epidemiologic studies in type 1A DM suggest the involvement of certain environmental factors in its pathogenesis, though role of none of them has been conclusively proved. In fact, the trigger may precede the occurrence of the disease by several years. It appears that certain viral and dietary proteins share antigenic properties with human cell surface proteins and trigger the immune attack on β -cells by a process of molecular mimicry. These factors include the following:

- Certain viral infections* preceding the onset of disease e.g. mumps, measles, coxsackie B virus, cytomegalovirus and infectious mononucleosis.
- Experimental induction* of type 1A DM with certain chemicals has been possible e.g. alloxan, streptozotocin and pentamidine.
- Geographic and seasonal variations* in its incidence suggest some common environmental factors.

iv) Possible relationship of early exposure to *bovine milk proteins* and occurrence of autoimmune process in type 1A DM is being studied.

SUMMARY: Pathogenesis of type 1A DM can be summed up by interlinking the above three factors as under:

1. At birth, individuals with *genetic susceptibility* to this disorder have normal β -cell mass.
2. Destruction of β -cell mass occurs by *autoimmune phenomena* and takes months to years. Clinical features of diabetes manifest after more than 80% of β -cell mass has been destroyed.
3. The trigger for autoimmune process appears to be some *infectious or environmental factor* which specifically targets β -cells.

PATHOGENESIS OF TYPE 2 DM. The basic metabolic defect in type 2 DM is either a delayed insulin secretion relative to glucose load (*impaired insulin secretion*), or the peripheral tissues are unable to respond to insulin (*insulin resistance*).

Type 2 DM is a heterogeneous disorder with a more complex etiology and is far more common than type 1, but much less is known about its pathogenesis. A number of factors have been implicated though, but HLA association and autoimmune phenomena are not implicated. These factors are as under (Fig. 31.2,B):

1. Genetic factors. Genetic component has a stronger basis for type 2 DM than type 1A DM. Although no definite and consistent genes have been identified, multifactorial inheritance is the most important factor in development of type 2 DM:

- i) There is approximately 80% chance of developing diabetes in the other *identical twin* if one twin has the disease.
- ii) A person with one parent having type 2 DM is at an increased risk of getting diabetes, but if *both parents* have type 2 DM the risk in the offspring rises to 40%.

2. Constitutional factors. Certain environmental factors such as obesity, hypertension, and level of physical activity play contributory role and modulate the phenotyping of the disease.

3. Insulin resistance. One of the most prominent metabolic features of type 2 DM is the lack of responsiveness of peripheral tissues to insulin, especially of skeletal muscle and liver. Obesity, in particular, is strongly associated with insulin resistance and hence type 2 DM. Mechanism of hyperglycaemia in these cases is explained as under:

- i) Resistance to action of insulin *impairs glucose utilisation* and hence hyperglycaemia.

ii) There is *increased hepatic synthesis* of glucose.

iii) *Hyperglycaemia in obesity* is related to high levels of free fatty acids and cytokines (e.g. TNF- α and adiponectin) affect peripheral tissue sensitivity to respond to insulin.

The precise underlying molecular defect responsible for insulin resistance in type 2 DM has yet not been fully identified. Currently, it is proposed that insulin resistance may be possibly due to one of the following defects:

■ Polymorphism in various *post-receptor intracellular signal pathway molecules*.

■ *Elevated free fatty acids* seen in obesity may contribute e.g. by impaired glucose utilisation in the skeletal muscle, by increased hepatic synthesis of glucose, and by impaired β -cell function.

Insulin resistance syndrome is a complex of clinical features occurring from insulin resistance and its resultant metabolic derangements that includes hyperglycaemia and compensatory hyperinsulinaemia. The clinical features are in the form of accelerated cardiovascular disease and may occur in both obese as well as non-obese type 2 DM patients. The features include: mild hypertension (related to endothelial dysfunction) and dyslipidaemia (characterised by reduced HDL level, increased triglycerides and LDL level).

4. Impaired insulin secretion. In type 2 DM, insulin resistance and insulin secretion are interlinked:

- i) Early in the course of disease, in response to insulin resistance there is compensatory increased secretion of insulin (*hyperinsulinaemia*) in an attempt to maintain normal blood glucose level.
- ii) Eventually, however, there is *failure of β -cell function* to secrete adequate insulin, although there is some secretion of insulin i.e. cases of type 2 DM have mild to moderate deficiency of insulin (which is much less severe than that in type 1 DM) but not its total absence.

The exact genetic mechanism why there is a fall in insulin secretion in these cases is unclear. However, following possibilities are proposed:

■ Islet amyloid polypeptide (*amylin*) which forms fibrillar protein deposits in pancreatic islets in longstanding cases of type 2 DM may be responsible for impaired function of β -cells islet cells.

■ Metabolic environment of chronic hyperglycaemia surrounding the islets (*glucose toxicity*) may paradoxically impair islet cell function.

■ Elevated free fatty acid levels (*lipotoxicity*) in these cases may worsen islet cell function.

5. Increased hepatic glucose synthesis. One of the normal roles played by insulin is to promote hepatic storage of glucose as glycogen and suppress gluconeogenesis. In type 2 DM, as a part of insulin resistance by peripheral tissues, liver also shows insulin resistance i.e. in spite of hyperinsulinaemia in the early stage of disease, gluconeogenesis in the liver is not suppressed. This results in increased hepatic synthesis of glucose which contributes to hyperglycaemia in these cases.

SUMMARY: In essence, hyperglycaemia in type 2 DM is not due to destruction of β -cells but is instead a failure of β -cells to meet the requirement of insulin in the body. Its pathogenesis can be summed up by interlinking the above factors as under:

1. Type 2 DM is a more *complex multifactorial disease*.
2. There is greater role of *genetic defect and heredity*.
3. The two main mechanisms for hyperglycaemia in type 2 DM—*insulin resistance and impaired insulin secretion*, are interlinked.
4. While *obesity* plays a role in pathogenesis of insulin resistance, impaired insulin secretion may be from many constitutional factors.
5. *Increased hepatic synthesis of glucose* in initial period of disease contributes to hyperglycaemia.

PATHOLOGIC CHANGES IN PANCREATIC ISLETS

Pathologic changes in islets have been demonstrated in both types of diabetes, though the changes are more distinctive in type 1 DM.

1. INSULITIS: In type 1 DM, characteristically, in early stage there is lymphocytic infiltrate, mainly by T cells, in the islets which may be accompanied by a few macrophages and polymorphs. Diabetic infants born to diabetic mothers, however, have eosinophilic infiltrate in the islets.

In type 2 DM, there is no significant leucocytic infiltrate in the islets but there is variable degree of fibrous tissue in the islets.

2. ISLET CELL MASS: In type 1 DM, as the disease becomes chronic there is progressive depletion of β -cell mass, eventually resulting in total loss of pancreatic β -cells.

In type 2 DM, β -cell mass is either normal or mildly reduced. Infants of diabetic mothers, however, have hyperplasia and hypertrophy of islets as a compensatory response to maternal hyperglycaemia.

3. β -CELL DEGRANULATION: In type 1 DM, EM shows degranulation of remaining β -cells of islets.

In type 2 DM, no such change is observed.

4. AMYLOIDOSIS: In type 1 DM, deposits of amyloid around islets are absent.

In type 2 DM, characteristically chronic long-standing cases show deposition of amyloid material around the capillaries of the islets causing compression and atrophy of islet tissue.

CLINICAL FEATURES

It can be appreciated that hyperglycaemia in DM does not cause a single disease but is associated with numerous diseases and symptoms, especially due to complications. Two main types of DM can be distinguished clinically to the extent shown in Table 31.2. However, overlapping of clinical features occurs as regards the age of onset, duration of symptoms and family history. Pathophysiology in evolution of clinical features is schematically shown in Fig. 31.3.

Type 1 DM:

- i) Patients of type 1 DM usually manifest at early age, generally below the age of 35.
- ii) The onset of symptoms is often abrupt.
- iii) At presentation, these patients have polyuria, polydipsia and polyphagia.
- iv) The patients are not obese but have generally progressive loss of weight.
- v) These patients are prone to develop metabolic complications such as ketoacidosis and hypoglycaemic episodes.

Type 2 DM:

- i) This form of diabetes generally manifests in middle life or beyond, usually above the age of 40.
- ii) The onset of symptoms in type 2 DM is slow and insidious.
- iii) Generally, the patient is asymptomatic when the diagnosis is made on the basis of glucosuria or hyperglycaemia during physical examination, or may present with polyuria and polydipsia.
- iv) The patients are frequently obese and have unexplained weakness and loss of weight.
- v) Metabolic complications such as ketoacidosis are infrequent.

PATHOGENESIS OF COMPLICATIONS

It is now known that in both type 1 and 2 DM, *severity and chronicity of hyperglycaemia* forms the main

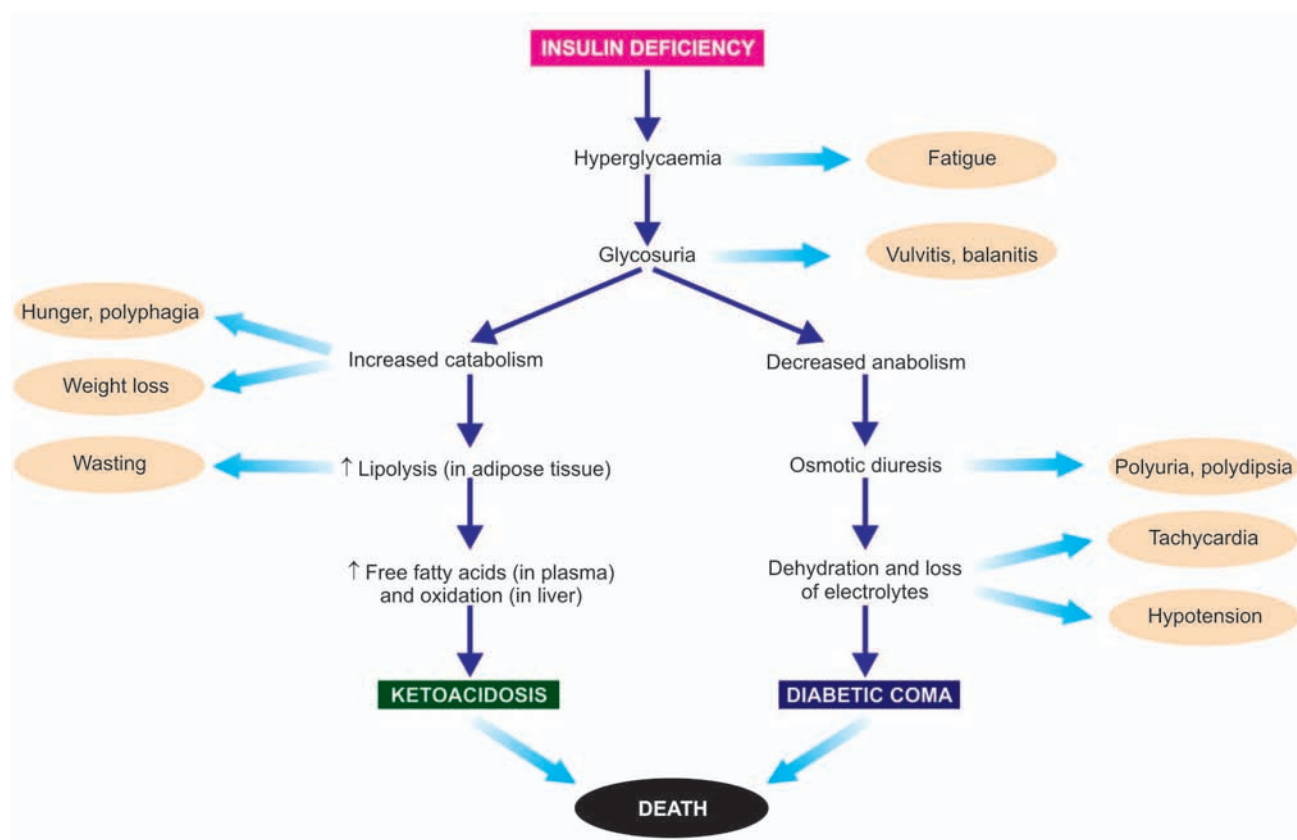


FIGURE 31.3

Pathophysiological basis of common signs and symptoms due to uncontrolled hyperglycaemia in diabetes mellitus.

pathogenetic mechanism for 'microvascular complications' (e.g. retinopathy, nephropathy, neuropathy); therefore control of blood glucose level constitutes the mainstay of treatment for minimising development of these complications. Longstanding cases of type 2 DM, however, in addition, frequently develop 'macrovascular complications' (e.g. atherosclerosis, coronary artery disease, peripheral vascular disease, cerebrovascular disease) which are more difficult to explain on the basis of hyperglycaemia alone.

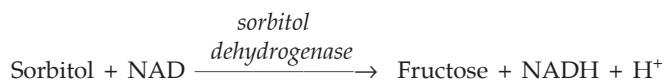
The following biochemical mechanisms have been proposed to explain the development of complications of diabetes mellitus (Fig. 31.4,A):

1. Non-enzymatic protein glycosylation: The free amino group of various body proteins binds by non-enzymatic mechanism to glucose; this process is called *glycosylation* and is directly proportionate to the severity of hyperglycaemia. Various body proteins undergoing chemical alterations in this way include haemoglobin, lens crystalline protein, and basement membrane of body cells. An example is the measurement of a fraction

of haemoglobin called glycosylated haemoglobin (HbA_{1c}) as a test for monitoring glycaemic control in a diabetic patient during the preceding 100 to 120 days which is lifespan of red cells. Similarly, there is accumulation of labile and reversible glycosylation products on collagen and other tissues of the blood vessel wall which subsequently become stable and irreversible by chemical changes and form advanced glycosylation end-products (AGE). The AGEs bind to receptors on different cells and produce a variety of biologic and chemical changes e.g. thickening of vascular basement membrane in diabetes.

2. Polyol pathway mechanism: This mechanism is responsible for producing lesions in the aorta, lens of the eye, kidney and peripheral nerves. These tissues have an enzyme, aldose reductase, that reacts with glucose to form sorbitol and fructose in the cells of the hyperglycaemic patient as under:





Intracellular accumulation of sorbitol and fructose so produced results in entry of water inside the cell and consequent cellular swelling and cell damage. Also, intracellular accumulation of sorbitol causes intracellular deficiency of myoinositol which promotes injury to Schwann cells and retinal pericytes. These polyols result in disturbed processing of normal intermediary metabolites leading to complications of diabetes.

3. Excessive oxygen free radicals. In hyperglycaemia, there is increased production of reactive oxygen free radicals from mitochondrial oxidative phosphorylation which may damage various target cells in diabetes.

COMPLICATIONS OF DIABETES MELLITUS

As a consequence of hyperglycaemia of diabetes, every tissue and organ of the body undergoes biochemical and structural alterations which account for the major complications in diabetics which may be *acute metabolic* or *chronic systemic*.

Both types of diabetes mellitus may develop complications which are broadly divided into 2 major groups:

I. *Acute metabolic complications:* These include diabetic ketoacidosis, hyperosmolar nonketotic coma, and hypoglycaemia.

II. *Late systemic complications:* These are atherosclerosis, diabetic microangiopathy, diabetic nephropathy, diabetic neuropathy, diabetic retinopathy and infections.

I. Acute Metabolic Complications

Metabolic complications develop acutely. While ketoacidosis and hypoglycaemic episodes are primarily complications of type 1 DM, hyperosmolar nonketotic coma is chiefly a complication of type 2 DM (also see Fig. 31.3).

1. DIABETIC KETOACIDOSIS. Ketoacidosis is almost exclusively a complication of type 1 DM. It can develop in patients with severe insulin deficiency combined with glucagon excess. Failure to take insulin and exposure to stress are the usual precipitating causes. Severe lack of insulin causes lipolysis in the adipose tissues, resulting in release of free fatty acids into the plasma. These free fatty acids are taken up by the liver where they are oxidised through acetyl coenzyme-A to ketone bodies, principally acetoacetic acid and β -hydroxybutyric acid. Such free fatty acid oxidation to ketone bodies is accelerated in the presence of elevated level of glucagon. Once the rate of ketogenesis exceeds the rate at which the ketone bodies can be utilised by the

muscles and other tissues, ketonaemia and ketonuria occur. If urinary excretion of ketone bodies is prevented due to dehydration, systemic metabolic ketoacidosis occurs. Clinically, the condition is characterised by anorexia, nausea, vomitings, deep and fast breathing, mental confusion and coma. Most patients of ketoacidosis recover.

2. HYPEROSMOLAR NONKETOTIC COMA.

Hyperosmolar nonketotic coma is usually a complication of type 2 DM. It is caused by severe dehydration resulting from sustained hyperglycaemic diuresis. The loss of glucose in urine is so intense that the patient is unable to drink sufficient water to maintain urinary fluid loss. The usual clinical features of ketoacidosis are absent but prominent central nervous signs are present. Blood sugar is extremely high and plasma osmolality is high. Thrombotic and bleeding complications are frequent due to high viscosity of blood. The mortality rate in hyperosmolar nonketotic coma is high.

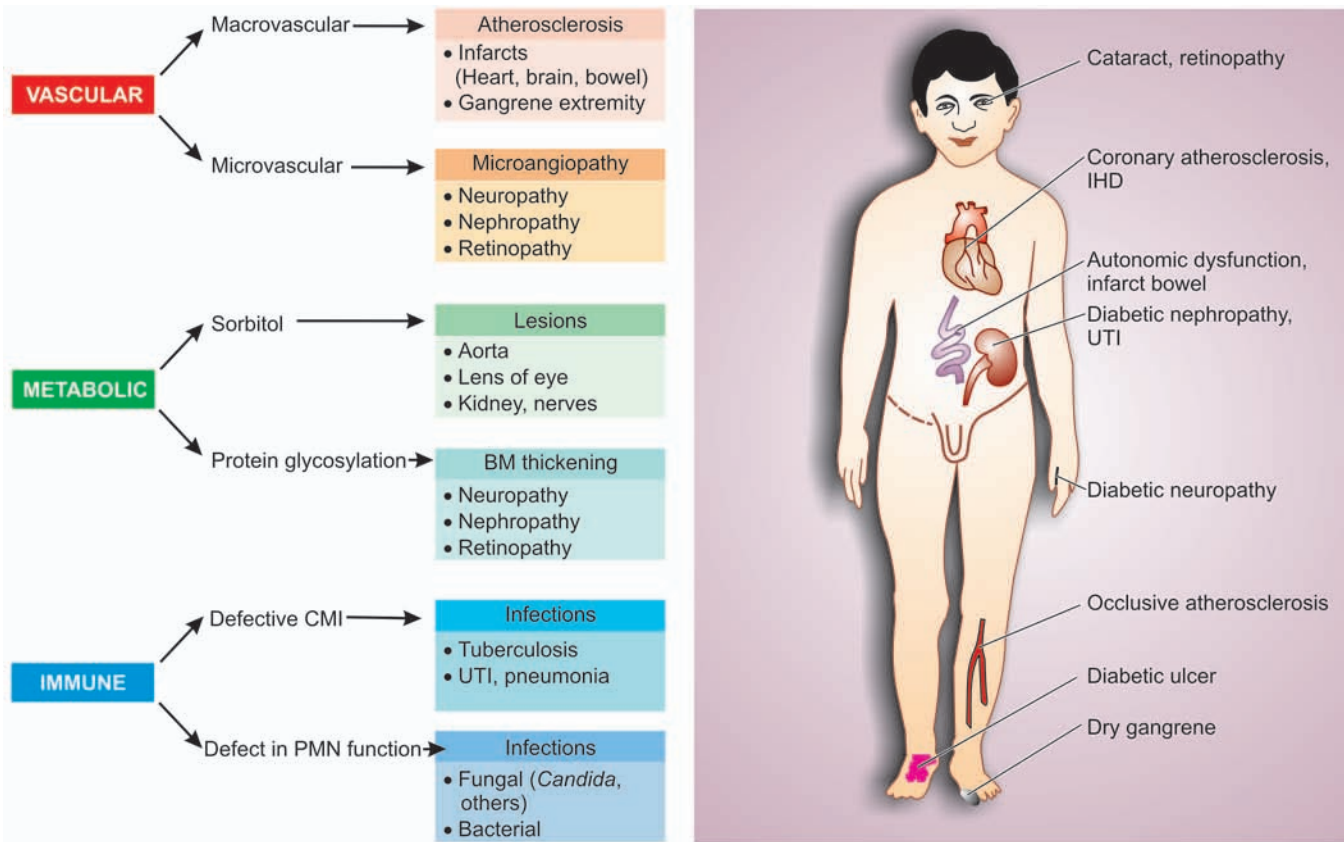
3. HYPOGLYCAEMIA. Hypoglycaemic episode may develop in patients of type 1 DM. It may result from excessive administration of insulin, missing a meal, or due to stress. Hypoglycaemic episodes are harmful as they produce permanent brain damage, or may result in worsening of diabetic control and rebound hyperglycaemia, so called *Somogyi's effect*.

II. Late Systemic Complications

A number of systemic complications may develop after a period of 15-20 years in either type of diabetes. These late complications are largely responsible for morbidity and premature mortality in diabetes mellitus. These complications are briefly outlined below as they are discussed in detail in relevant chapters (Fig. 31.4,B).

1. ATHEROSCLEROSIS. Diabetes mellitus of both type 1 and type 2 accelerates the development of atherosclerosis so that consequent atherosclerotic lesions appear earlier than in the general population, are more extensive, and are more often associated with complicated plaques such as ulceration, calcification and thrombosis (page 305). The cause for this accelerated atherosclerotic process is not known but possible contributory factors are hyperlipidaemia, reduced HDL levels, nonenzymatic glycosylation, increased platelet adhesiveness, obesity and associated hypertension in diabetes.

The possible ill-effects of accelerated atherosclerosis in diabetes are early onset of coronary artery disease, silent myocardial infarction, cerebral stroke and gangrene of the toes and feet. Gangrene of the lower extremities is 100 times more common in diabetics than in non-diabetics.



A. PATHOGENESIS OF LONG TERM COMPLICATIONS OF DIABETES B. SECONDARY SYSTEMIC COMPLICATIONS OF DIABETES

FIGURE 31.4

Long-term complications of diabetes mellitus. A, Pathogenesis. B, Secondary systemic complications.

2. DIABETIC MICROANGIOPATHY. Microangiopathy of diabetes is characterised by basement membrane thickening of small blood vessels and capillaries of different organs and tissues such as the skin, skeletal muscle, eye and kidney. Similar type of basement membrane-like material is also deposited in nonvascular tissues such as peripheral nerves, renal tubules and Bowman's capsule. The pathogenesis of diabetic microangiopathy as well as of peripheral neuropathy in diabetics is believed to be due to recurrent hyperglycaemia that causes *increased glycosylation* of haemoglobin and other proteins (e.g. collagen and basement membrane material) resulting in thickening of basement membrane.

3. DIABETIC NEPHROPATHY. Renal involvement is an important complication of diabetes mellitus. End-stage kidney with renal failure accounts for deaths in more than 10% of all diabetics. Renal complications are more severe, develop early and more frequently in

type 1 (i.e. insulin-dependent) diabetes mellitus (30-40% cases) than in type 2 (non-insulin-dependent) diabetics (about 20% cases). A variety of clinical syndromes are associated with diabetic nephropathy that includes asymptomatic proteinuria, nephrotic syndrome, progressive renal failure and hypertension. Cardiovascular disease is 40 times more common in patients of end-stage renal disease in diabetes mellitus than in non-diabetics and more diabetics die from cardiovascular complications than from uraemia.

PATHOLOGIC CHANGES. Diabetic nephropathy is the term that encompasses 4 types of renal lesions in diabetes mellitus: diabetic glomerulosclerosis, vascular lesions, diabetic pyelonephritis and tubular lesions (Armanni-Ebstein lesions).

A. DIABETIC GLOMERULOSCLEROSIS. Glomerular lesions in diabetes mellitus are particularly

common and account for majority of abnormal findings referable to the kidney.

Pathogenesis of these lesions in diabetes mellitus is explained by following sequential changes: hyperglycaemia → glomerular hypertension → renal hyperperfusion → deposition of proteins in the mesangium → glomerulosclerosis → renal failure. In addition, cellular infiltration in renal lesions in diabetic glomerular lesions is due to growth factors, particularly transforming growth factor- β . Strict control of blood glucose level and control of systemic hypertension in these patients retards progression to diabetic nephropathy.

Glomerulosclerosis in diabetes may take one of the 2 forms: diffuse or nodular lesions.

i) Diffuse glomerulosclerosis. Diffuse glomerular lesions are the most common. There is involvement of all parts of glomeruli. The pathologic changes consist of thickening of the GBM and diffuse increase in mesangial matrix with mild proliferation of mesangial cells. Various exudative lesions such as capsular hyaline drops and fibrin caps may also be present (Fig. 31.5,A) *Capsular drop* is an eosinophilic hyaline thickening of the parietal layer of Bowman's capsule and bulges into the glomerular space. *Fibrin*

cap is homogeneous, brightly eosinophilic material appearing on the wall of a peripheral capillary of a lobule.

ii) Nodular glomerulosclerosis. Nodular lesions of diabetic glomerulosclerosis are also called as *Kimmelstiel-Wilson (KW) lesions* or *intercapillary glomerulosclerosis*. These lesions are specific for juvenile-onset diabetes or islet cell antibody-positive diabetes mellitus. The pathologic changes consist of one or more nodules in a few or many glomeruli. *Nodule* is an ovoid or spherical, laminated, hyaline, acellular mass located within a lobule of the glomerulus. The nodules are surrounded peripherally by glomerular capillary loops which may have normal or thickened GBM (Fig. 31.5,B). The nodules are PAS-positive and contain lipid and fibrin. As the nodular lesions enlarge, they compress the glomerular capillaries and obliterate the glomerular tuft. As a result of glomerular and arteriolar involvement, renal ischaemia occurs leading to tubular atrophy and interstitial fibrosis and grossly small, contracted kidney.

B. VASCULAR LESIONS. *Atheroma* of renal arteries is very common and severe in diabetes mellitus. *Hyaline arteriosclerosis* affecting the afferent and efferent arterioles of the glomeruli is also often severe

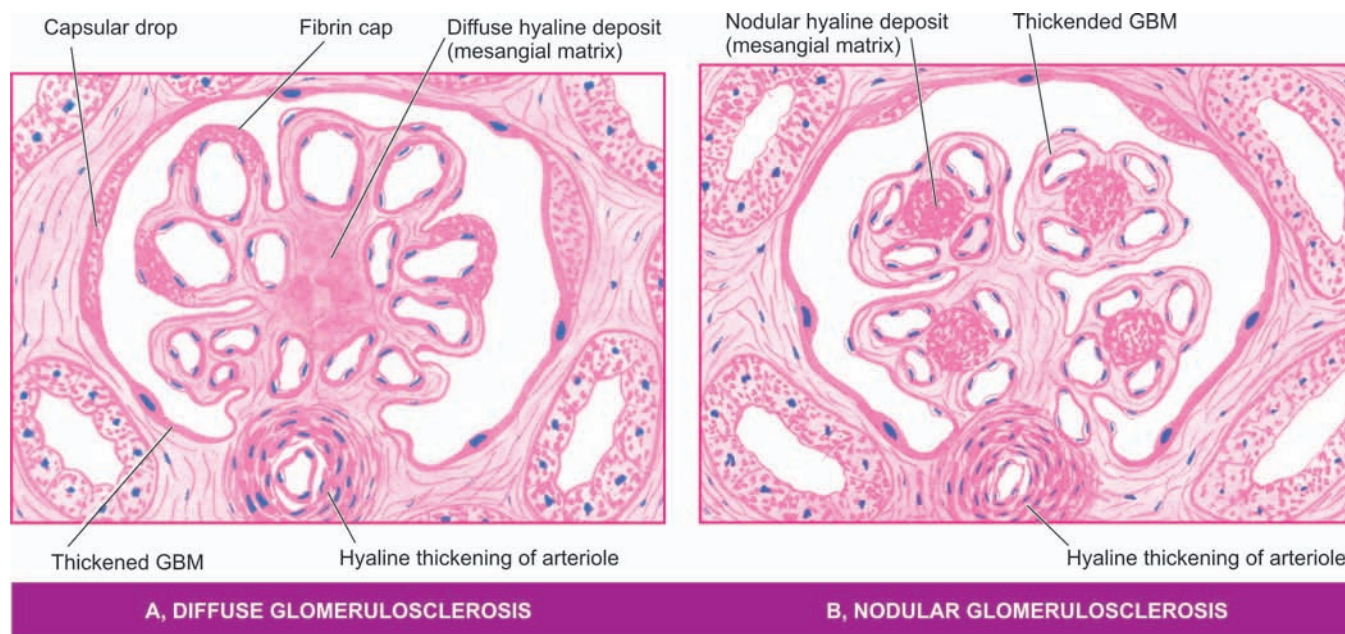


FIGURE 31.5

Diabetic glomerulosclerosis, microscopic appearance. *A, Diffuse lesions.* The characteristic features are diffuse involvement of the glomeruli showing thickening of the GBM and diffuse increase in the mesangial matrix with mild proliferation of mesangial cells and exudative lesions (fibrin caps and capsular drops). *B, Nodular lesion (Kimmelstiel-Wilson Lesion).* There are one or more hyaline nodules within the lobules of glomeruli, surrounded peripherally by glomerular capillaries with thickened walls.

in diabetes. These vascular lesions are responsible for renal ischaemia that results in tubular atrophy and interstitial fibrosis.

C. DIABETIC PYELONEPHRITIS. Poorly-controlled diabetics are particularly susceptible to bacterial infections. Papillary necrosis (necrotising papillitis) is an important complication of diabetes that may result in acute pyelonephritis. Chronic pyelonephritis is 10 to 20 times more common in diabetics than in others.

D. TUBULAR LESIONS (ARMANNI-EBSTEIN LESIONS). In untreated diabetics who have extremely high blood sugar level, the epithelial cells of the proximal convoluted tubules develop extensive glycogen deposits appearing as vacuoles. These are called Armanni-Ebstein lesions. The tubules return to normal on control of hyperglycaemic state.

4. DIABETIC NEUROPATHY. Diabetic neuropathy may affect all parts of the nervous system but symmetric peripheral neuropathy is most characteristic. The basic pathologic changes are segmental demyelination, Schwann cell injury and axonal damage. The pathogenesis of neuropathy is not clear but it may be related to diffuse microangiopathy as already explained, or may be due to accumulation of sorbitol and fructose as a result of hyperglycaemia, leading to deficiency of myoinositol.

5. DIABETIC RETINOPATHY. Diabetic retinopathy is an important cause of blindness. It is related to the degree and duration of glycaemic control. The condition develops in more than 60% of diabetics 15 years or so after the onset of disease, and in about 2% of diabetics causes blindness. Other ocular complications of diabetes include glaucoma, cataract and corneal disease. Most cases of diabetic retinopathy occur over the age of 50 years. The risk is greater in type 1 diabetes mellitus (IDDM) than in type 2 diabetes mellitus, although in clinical practice there are more patients of diabetic retinopathy due to type 2 diabetes mellitus because of its higher prevalence. Women are more prone to diabetes as well as diabetic retinopathy. Diabetic retinopathy is directly correlated with Kimmelstiel-Wilson nephropathy.

Microscopically, two types of changes are described in diabetic retinopathy—background (non-proliferative) and proliferative retinopathy.

1. Background (non-proliferative) retinopathy. This is the initial retinal capillary microangiopathy. The following changes are seen:

- i) Basement membrane shows varying thickness due to increased synthesis of basement membrane substance.
- ii) Degeneration of pericytes and some loss of endothelial cells are found.
- iii) Capillary microaneurysms appear which may develop thrombi and get occluded.
- iv) 'Waxy exudates' accumulate in the vicinity of microaneurysms especially in the elderly diabetics because of hyperlipidaemia.
- v) 'Dot and blot haemorrhages' in the deeper layers of retina are produced due to diapedesis of erythrocytes.
- vi) Soft 'cotton-wool spots' appear on the retina which are microinfarcts of nerve fibre layers. 'Scotomas' appear from degeneration of nerve fibres and ganglion cells.

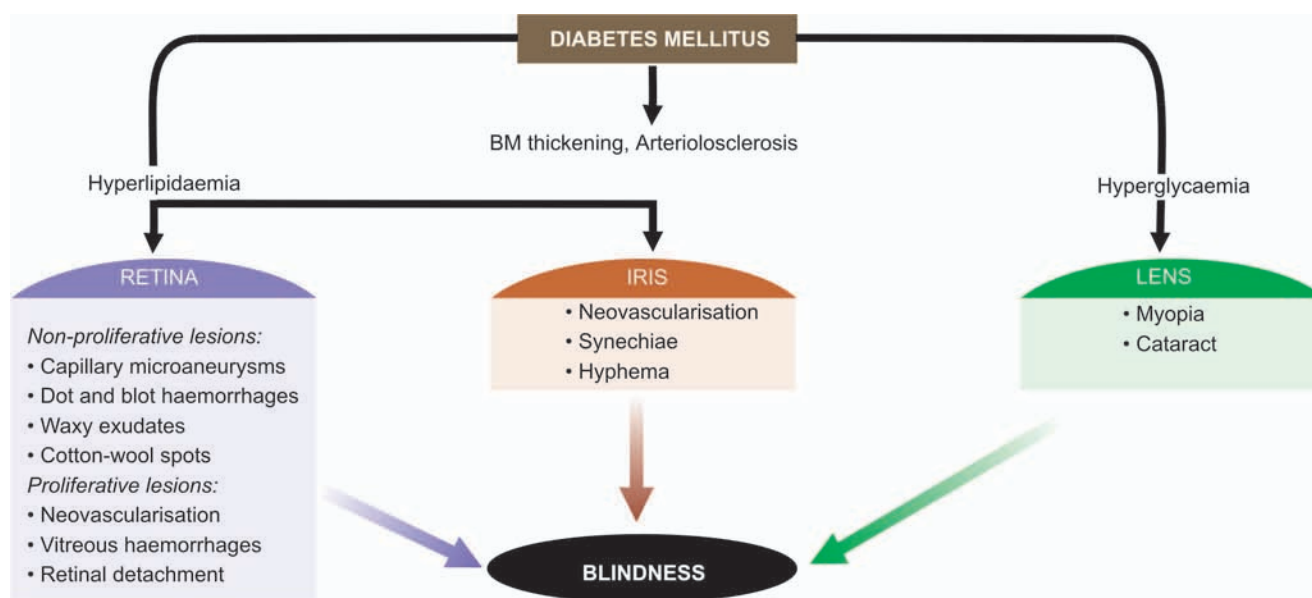
2. Proliferative retinopathy (retinitis proliferans). After many years, retinopathy becomes proliferative. Severe ischaemia and chronic hypoxia for long period leads to secretion of angiogenic factor by retinal cells and results in the following changes:

- i) Neovascularisation of the retina at the optic disc.
- ii) Friability of newly-formed blood vessels causes them to bleed easily and results in vitreous haemorrhages.
- iii) Proliferation of astrocytes and fibrous tissue around the new blood vessels.
- iv) Fibrovascular and gliotic tissue contracts to cause retinal detachment and blindness.

In addition to the changes on retina, severe diabetes may cause diabetic iridopathy with formation of adhesions between iris and cornea (peripheral anterior synechiae) and between iris and lens (posterior synechiae). Diabetics also develop cataract of the lens at an earlier age than the general population.

The pathogenesis of blindness in diabetes mellitus is schematically outlined in Fig. 31.6.

6. INFECTIONS. Diabetics have enhanced susceptibility to various infections such as tuberculosis, pneumonias, pyelonephritis, otitis, carbuncles and diabetic ulcers. This could be due to various factors such as impaired leucocyte functions, reduced cellular immunity, poor blood supply due to vascular involvement and hyperglycaemia *per se*.

**FIGURE 31.6**

The effects of diabetes mellitus on eye in causing blindness.

DIAGNOSIS OF DIABETES

Hyperglycaemia remains the fundamental basis for the diagnosis of diabetes mellitus. In *symptomatic cases*, the diagnosis is not a problem and can be confirmed by finding glucosuria and a random plasma glucose concentration above 200 mg/dl (Fig. 31.7,D).

■ The severity of clinical symptoms of polyuria and polydipsia is directly related to the degree of hyperglycaemia.

■ In *asymptomatic cases*, when there is persistently elevated fasting plasma glucose level, diagnosis again poses no difficulty.

■ The problem arises in asymptomatic patients who have normal fasting glucose level in the plasma but are suspected to have diabetes on other grounds and are thus subjected to oral glucose tolerance test (GTT). If abnormal GTT values are found, these subjects are said to have 'chemical diabetes'. The WHO-American Diabetes Association (2000) have suggested definite diagnostic criteria for early diagnosis of diabetes mellitus (Table 31.3).

The following investigations are helpful in establishing the diagnosis of diabetes mellitus:

I. URINE TESTING. Urine tests are cheap and convenient but the diagnosis of diabetes cannot be based on urine testing alone since there may be false-positives

and false-negatives. They can be used in population screening surveys. Urine is tested for the presence of glucose and ketones.

1. Glucosuria. *Benedict's qualitative test* detects any reducing substance in the urine and is not specific for glucose. More sensitive and glucose specific test is *dipstick* method based on enzyme-coated paper strip which turns purple when dipped in urine containing glucose.

The main disadvantage of relying on urinary glucose test alone is the individual variation in renal threshold. Thus, a diabetic patient may have a negative urinary glucose test and a nondiabetic individual with low renal threshold may have a positive urine test.

Besides diabetes mellitus, *glucosuria* may also occur in certain other conditions such as: renal glycosuria, alimentary (lag storage) glucosuria, many metabolic disorders, starvation and intracranial lesions (e.g. cerebral tumour, haemorrhage and head injury). However, two of these conditions—renal glycosuria and alimentary glucosuria, require further elaboration here.

■ *Renal glycosuria* (Fig. 31.7,B): After diabetes, the next most common cause of glucosuria is the reduced renal threshold for glucose. In such cases although the blood glucose level is below 180 mg/dl (i.e. below normal renal threshold for glucose) but glucose still appears regularly and consistently in the urine due to lowered renal threshold.

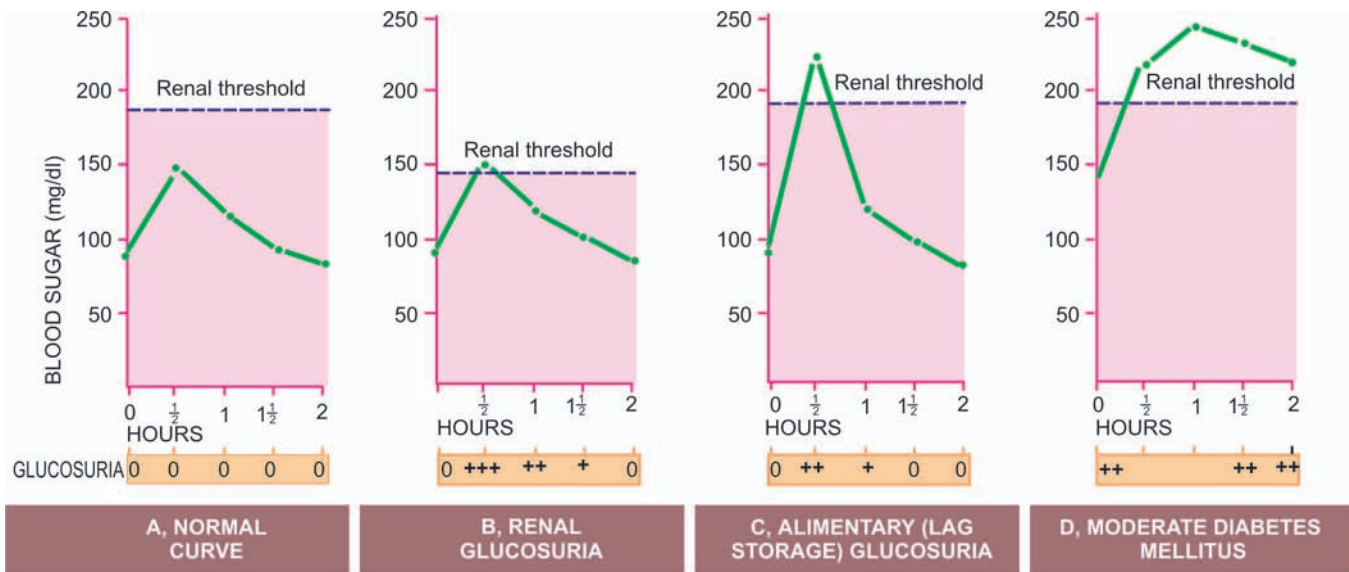


FIGURE 31.7

The glucose tolerance test, showing blood glucose curves (venous blood glucose) and glucosuria after 75 gm of oral glucose.

Renal glucosuria is a benign condition unrelated to diabetes and runs in families and may occur temporarily in pregnancy without symptoms of diabetes.

■ **Alimentary (lag storage) glucosuria** (Fig. 31.7,C): A rapid and transitory rise in blood glucose level above the normal renal threshold may occur in some individuals after a meal. During this period, glucosuria is present. This type of response to meal is called 'lag storage curve' or more appropriately 'alimentary glucosuria'. A characteristic feature is that unusually high blood glucose level returns to normal 2 hours after meal.

2. Ketonuria. Tests for ketone bodies in the urine are required for assessing the severity of diabetes and not for diagnosis of diabetes. However, if both glucosuria

and ketonuria are present, diagnosis of diabetes is almost certain. *Rothera's test* (nitroprusside reaction) and *strip test* are conveniently performed for detection of ketonuria.

Besides uncontrolled diabetes, ketonuria may appear in individuals with prolonged vomitings, fasting state or exercising for long periods.

II. SINGLE BLOOD SUGAR ESTIMATION. For diagnosis of diabetes, blood sugar determinations are absolutely necessary. *Folin-Wu method* of measurement of all reducing substances in the blood including glucose is now obsolete. Currently used are *O-toluidine*, *Somogyi-Nelson* and *glucose oxidase* methods. Whole blood or plasma may be used but *whole blood values* are 15% lower than *plasma values*.

TABLE 31.3: Revised Criteria for Diagnosis of Diabetes by Oral GTT (as per WHO-American Diabetes Association, 2000).

PATIENT STATUS	PLASMA GLUCOSE VALUE*	DIAGNOSIS
1. Fasting value	below 110 mg/dl (< 6.1 mmol/L)	Normal fasting value
2. Fasting value	110-126 mg/dl (6.1-7.0 mmol/L)	Impaired fasting glucose (IFG)**
3. Fasting value	126 mg/dl (7.0 mmol/L) or more	Diabetes mellitus
4. Two-hour after 75 g oral glucose load	140-200 mg/dl (7.8-11.1 mmol/L)	Impaired glucose tolerance (IGT)**
5. Two-hour after 75 g oral glucose load	200 mg/dl (11.1 mmol/L) or more	Diabetes mellitus
6. Random value	200 mg/dl (11.1 mmol/L) or more in a symptomatic patient	Diabetes mellitus

Note: * Plasma glucose values are 15% higher than whole blood glucose value.

** Individuals with IFG and IGT are at increased risk for development of type 2 DM later.

A grossly elevated single determination of plasma glucose may be sufficient to make the diagnosis of diabetes. A *fasting plasma glucose value above 126 mg/dl (7 mmol/L) is certainly indicative of diabetes*. In other cases, oral GTT is performed (Fig. 31.7).

III. ORAL GLUCOSE TOLERANCE TEST. The patient who is scheduled for oral GTT is instructed to eat a high carbohydrate diet for at least 3 days prior to the test and come after an overnight fast on the day of the test (for at least 8 hours). A fasting blood sugar sample is first drawn. Then 75 gm of glucose dissolved in 300 ml of water is given. Blood and urine specimen are collected at half-hourly intervals for at least 2 hours. Blood or plasma glucose content is measured and urine is tested for glucosuria to determine the approximate renal threshold for glucose. Venous whole blood concentrations are 15% lower than plasma glucose values.

Currently accepted criteria for diagnosis of DM (as per WHO-American Diabetes Association, 2000) are given in Table 31.3.

■ Individuals with fasting value of plasma glucose higher than 126 mg/dl and 2-hour value after 75 gm oral glucose higher than 200 mg/dl are labelled as *diabetics* (Fig. 31.7,D).

■ In a symptomatic case the random blood glucose value above 200 mg/dl is diagnosed as diabetes mellitus.

■ Normal cut off value for fasting blood glucose level is considered as 110 mg/dl. Cases with fasting blood glucose value between 110 and 126 mg/dl are considered as *impaired fasting glucose tolerance (IGT)*; these cases are at increased risk of developing diabetes later and

therefore kept under observation for repeating the test. During pregnancy, however, a case of IGT is treated as a diabetic.

IV. OTHER TESTS. A few other tests are sometimes performed in specific conditions in diabetics and for research purposes:

1. Glycosylated haemoglobin (HbA_{1C}). Measurement of blood glucose level in diabetics suffers from variation due to dietary intake of the previous day. Long-term objective assessment of degree of diabetic control is better done by measurement of glycosylated haemoglobin (HbA_{1C}), a minor haemoglobin component present in normal persons. This is because the non-enzymatic glycosylation of haemoglobin takes place over 120 days, life span of red blood cells. HbA_{1C} assay, therefore, gives an estimate of diabetic control for the preceding 6-10 weeks.

2. Extended GTT. The oral GTT is extended to 3-4 hours for appearance of symptoms of hyperglycaemia. It is a useful test in cases of reactive hypoglycaemia of early diabetes.

3. Intravenous GTT. This test is performed in persons who have intestinal malabsorption or in postgastrectomy cases.

4. Cortisone-primed GTT. This provocative test is a useful investigative aid in cases of potential diabetics.

5. Insulin assay. Plasma insulin levels can be measured by radioimmunoassay and ELISA techniques.

6. C-peptide assay. This test is even more sensitive than insulin assay because its levels are not affected by insulin therapy.



NORMAL STRUCTURE

The skeleton consists of cartilage and bone. Cartilage has a role in growth and repair of bone, and in the adults forms the articular skeleton responsible for movement of joints. Bone is a specialised form of connective tissue which performs the function of providing mechanical support and is also a mineral reservoir for calcium homeostasis. There are 206 bones in the human body, and depending upon their size and shape may be long, flat, tubular etc.

NORMAL STRUCTURE OF BONE

Bone is divided into 2 components (Fig. 32.1):

■ **Cortical or compact bone** comprises 80% of the skeleton and is the dense outer shell responsible for structural rigidity. It consists of haversian canals with blood vessels surrounded by concentric layers of mineralised collagen forming osteons which are joined together by cement lines (Fig. 32.1,A).

■ **Trabecular or cancellous bone** composes 20% of the skeleton and has trabeculae traversing the marrow space (Fig. 32.1,B). Its main role is in mineral homeostasis.

HISTOLOGY. Bone consists of large quantities of extracellular matrix which is loaded with calcium hydroxyapatite and relatively small number of bone cells which are of 3 main types: osteocytes, osteoblasts and osteoclasts, besides osteoid matrix.

1. Osteoblasts. Osteoblasts are uninucleate cells found abundantly along the new bone-forming surfaces. They synthesise bone matrix. The serum levels of bone-related *alkaline phosphatase* (other being hepatic alkaline phosphatase) is a marker for osteoblastic activity. Its levels are raised in puberty during period of active bone growth and in pathologic conditions associated with high osteoblastic activity such as in fracture repair and Paget's disease of the bone.

2. Osteocytes. Osteocytes are those osteoblasts which get incorporated into bone matrix during its synthesis. Osteocytes are found within small spaces called lacunae lying in the bone matrix. The distribution of the osteocytic lacunae is a reliable parameter for distinguishing between woven and lamellar bone.

■ **Woven bone** is immature and rapidly deposited bone containing large number of closely-packed osteocytes and consists of irregular interlacing pattern of

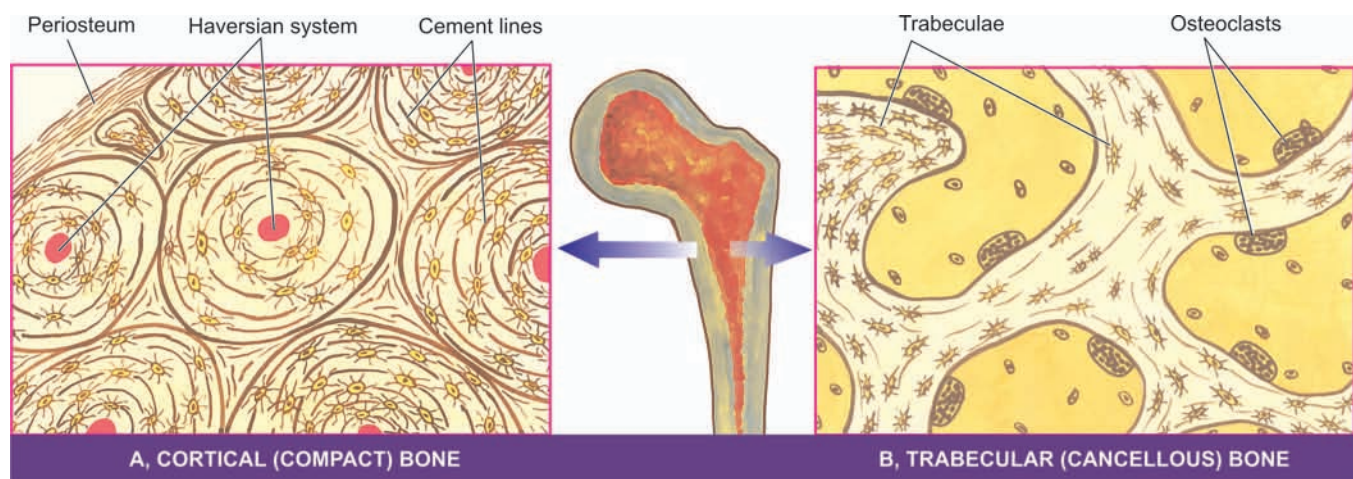


FIGURE 32.1

A, The normal structure of cortical (compact) bone and B, trabecular (cancellous) bone in transverse section. The cortical bone forming the outer shell shows concentric lamellae alongwith osteocytic lacunae surrounding central blood vessels, while the trabecular bone forming the marrow space shows trabeculae with osteoclastic activity at the margins.

collagen fibre bundles in bone matrix. Woven bone is seen in foetal life and in children under 4 years of age.

■ **Lamellar bone** differs from woven bone in having smaller and less numerous osteocytes and fine and parallel or lamellar sheets of collagen fibres. Lamellar bone usually replaces woven bone or pre-existing cartilage.

3. Osteoclasts. Osteoclasts are large multinucleate cells of mononuclear-macrophage origin and are responsible for bone resorption. The osteoclastic activity is determined by bone-related serum *acid phosphatase* levels (other being prostatic acid phosphatase). Osteoclasts are found along the endosteal surface of the cortical (compact) bone and the trabeculae of trabecular (cancellous) bone.

4. Osteoid matrix. The osteoid matrix of bone consists of 90-95% of collagen type I and comprises nearly half of total body's collagen. Virtually whole of body's hydroxyproline and hydroxylysine reside in the bone. The architecture of bone collagen reflects the rate of its synthesis and may be woven or lamellar, as described above.

BONE FORMATION AND RESORPTION. Bone is not a static tissue but its formation and resorption are taking place during period of growth as well as in adult life. Bone deposition is the result of osteoblasts while bone resorption is the function of osteoclasts. Bone formation may take place directly from collagen called *membranous ossification* seen in certain flat bones, or may occur through an intermediate stage of cartilage termed *endochondral ossification* found in metaphysis of long bones. In either case, firstly an uncalcified osteoid matrix is formed by osteoblasts which is then mineralised in 12-15 days. This delay in mineralisation results in formation of about 15 µm thick osteoid seams at calcification fronts (About > 1 µm of matrix osteoid is formed daily). Uncalcified osteoid appears eosinophilic in H & E stains and does not stain with von Kossa reaction, while mineralised osteoid is basophilic in appearance and stains black with von Kossa reaction (a stain for calcium). Areas of active bone resorption have scalloped edges of bone surface called Howship's lacunae and contain multinucleated osteoclasts. In this way, osteoblastic formation and osteoclastic resorption continue to take place into adult life in a balanced way termed *bone modelling*. There is important role of vitamin D₁, parathyroid hormone and calcitonin in calcium metabolism.

NORMAL STRUCTURE OF CARTILAGE

Unlike bone, the cartilage lacks blood vessels, lymphatics and nerves. It may have focal areas of calcification.

Cartilage consists of 2 components: cartilage matrix and chondrocytes.

■ **Cartilage matrix.** Like bone, cartilage too consists of organic and inorganic material. Inorganic material of cartilage is calcium hydroxyapatite similar to that in bone matrix but the organic material of cartilage is distinct from bone. It consists of very high content of water (80%) and remaining 20% consists of type II collagen and proteoglycans. High water content of cartilage matrix is responsible for function of articular cartilage and lubrication. Proteoglycans are macromolecules having proteins complexed with polysaccharides termed glycosaminoglycans. Cartilage glycosaminoglycans consist of chondroitin sulfate and keratan sulfate, the former being most abundant comprising 55-90% of cartilage matrix varying on the age of the cartilage.

■ **Chondrocytes.** Primitive mesenchymal cells which form bone cells form chondroblasts which give rise to chondrocytes. However, calcified cartilage is removed by the osteoclasts.

Depending upon location and structural composition, cartilage is of 3 types:

1. *Hyaline cartilage* is the basic cartilaginous tissue comprising articular cartilage of joints, cartilage in the growth plates of developing bones, costochondral cartilage, cartilage in the trachea, bronchi and larynx and the nasal cartilage. Hyaline cartilage is the type found in most cartilage-forming tumours and in the fracture callus.

2. *Fibrocartilage* is a hyaline cartilage that contains more abundant type II collagen fibres. It is found in annulus fibrosus of intervertebral disc, menisci, insertions of joint capsules, ligament and tendons. Fibrocartilage may also be found in some cartilage-forming tumours and in the fracture callus.

3. *Elastic cartilage* is hyaline cartilage that contains abundant elastin. Elastic cartilage is found in the pinna of ears, epiglottis and arytenoid cartilage of the larynx.

Diseases of skeletal system include infection (osteomyelitis), disordered growth and development (skeletal dysplasias), metabolic and endocrine derangements, and tumours and tumour-like conditions.

OSTEOMYELITIS

An infection of the bone is termed osteomyelitis (myelo = marrow). A number of systemic infectious diseases may spread to the bone such as enteric fever, actinomycosis, mycetoma (madura foot), syphilis, tuberculosis and brucellosis. However, two of the

conditions which produce significant pathologic lesions in the bone, namely pyogenic osteomyelitis and tuberculous osteomyelitis, are described below.

PYGENIC OSTEOMYELITIS

Suppurative osteomyelitis is usually caused by bacterial infection and rarely by fungi. Haematogenous pyogenic osteomyelitis occurs most commonly in the long bones of infants and young children (5-15 years of age), particularly in the developing countries of the world. In the developed world, however, where institution of antibiotics is early and prompt, haematogenous spread of infection to the bone is uncommon. In such cases, instead, direct extension of infection from the adjacent area, frequently involving the jaws and skull in adults, is more common mode of spread. Bacterial osteomyelitis may be a complication at all ages in patients with compound fractures, surgical procedures involving prosthesis or implants, gangrene of a limb in diabetics, debilitation and immunosuppression. Though any etiologic agent may cause osteomyelitis, *Staphylococcus aureus* is implicated in a vast majority of cases. Less frequently, other organisms such as streptococci, *Escherichia coli*, *Pseudomonas*, *Klebsiella* and anaerobes are involved. Mixed infections are common in post-traumatic cases of osteomyelitis. There may be transient bacteraemia preceding the development of osteomyelitis so that blood cultures may be positive.

Clinically, the child with acute haematogenous osteomyelitis has painful and tender limb. Fever, malaise

and leucocytosis generally accompany the bony lesion. Radiologic examination confirms the bony destruction.

Occasionally, osteomyelitis remains undiscovered until it becomes chronic. Draining sinus tracts may form which may occasionally be the site for development of squamous carcinoma. Persistence and chronicity of osteomyelitis over a period of time may lead to development of amyloidosis.

PATHOLOGIC CHANGES. Depending upon the duration, osteomyelitis may be *acute*, *subacute* or *chronic*. The basic pathologic changes in any stage of osteomyelitis are: suppuration, ischaemic necrosis, healing by fibrosis and bony repair. The *sequence of pathologic changes* is as under (Fig. 32.2):

1. The infection begins in the metaphyseal end of the *marrow cavity* which is largely occupied by *pus*. At this stage, microscopy reveals congestion, oedema and an exudate of neutrophils (**COLOUR PLATE XII: CL 45**).
2. The tension in the marrow cavity is increased due to pus and results in spread of infection along the marrow cavity, into the endosteum, and into the haversian and Volkmann's canal, causing *periosteitis*.
3. The infection may reach the subperiosteal space forming *subperiosteal abscesses*. It may penetrate through the cortex creating draining sinus tracts.
4. Combination of suppuration and impaired blood supply to the cortical bone results in erosion, thinning and infarction necrosis of the cortex called *sequestrum* (Fig. 32.3).

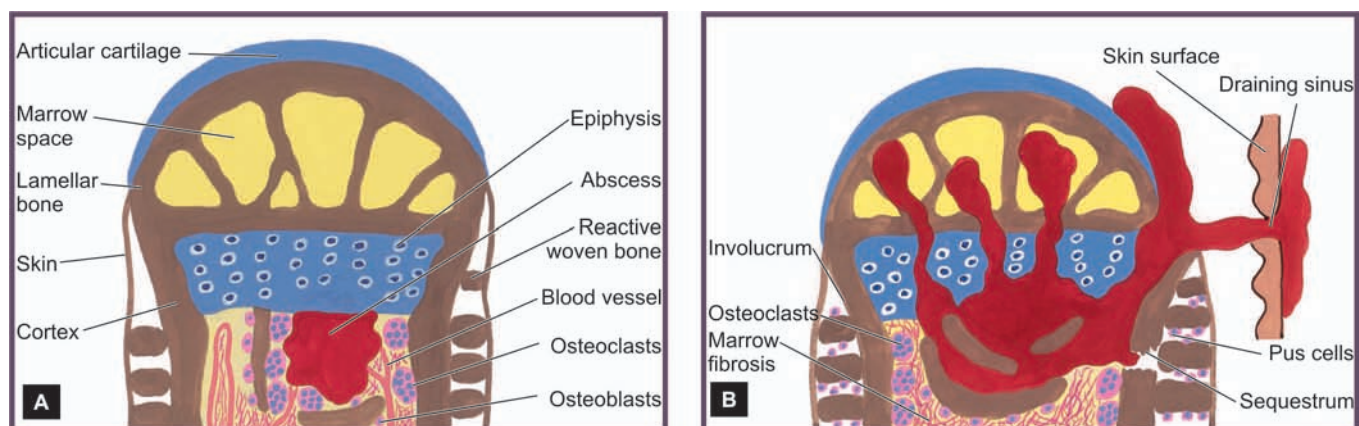


FIGURE 32.2

Pathogenesis of pyogenic osteomyelitis. A, The process begins as a focus of microabscess in a vascular loop in the marrow which expands to stimulate resorption of adjacent bony trabeculae. Simultaneously, there is beginning of reactive woven bone formation by the periosteum. B, The abscess expands further causing necrosis of the cortex called sequestrum. The formation of viable new reactive bone surrounding the sequestrum is called involucrum. The extension of infection into the joint space, epiphysis and the skin produces a draining sinus.

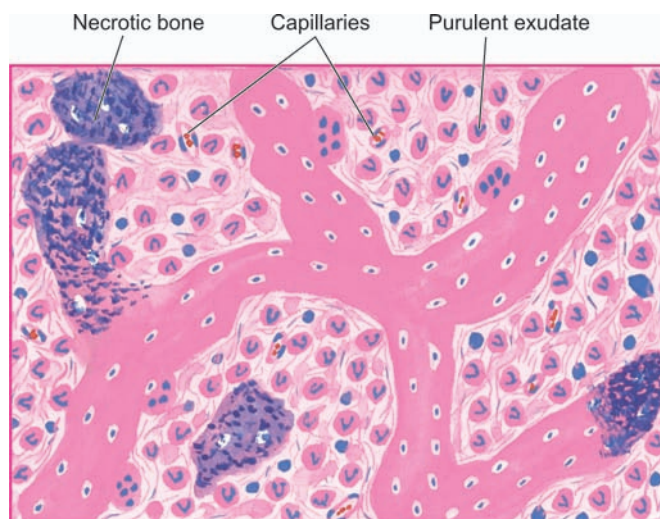


FIGURE 32.3

Chronic suppurative osteomyelitis. Histologic appearance shows necrotic bone and extensive purulent inflammatory exudate.

5. With passage of time, there is formation of new bone beneath the periosteum present over the infected bone. This forms an encasing sheath around the necrosed bone and is known as *involucrum*. Involucrum has irregular surface and has perforations through which discharging sinus tracts pass.
6. Long continued neo-osteogenesis gives rise to dense sclerotic pattern of osteomyelitis called *chronic sclerosing non-suppurative osteomyelitis of Garré*.
7. Occasionally, acute osteomyelitis may be contained to a localised area and walled off by fibrous tissue and granulation tissue. This is termed *Brodie's abscess*.
8. Occasionally, *vertebral osteomyelitis* may occur in which case infection begins from the disc (discitis) and spreads to involve the vertebral bodies (Fig. 32.4,A).

COMPLICATIONS. Osteomyelitis may result in the following complications:

1. Septicaemia.
2. Acute bacterial arthritis.
3. Pathologic fractures.
4. Development of squamous cell carcinoma in long-standing cases.
5. Secondary amyloidosis in long-standing cases.
6. Vertebral osteomyelitis may cause vertebral collapse with paravertebral abscess, epidural abscess, cord compression and neurologic deficits.

TUBERCULOUS OSTEOMYELITIS

Tuberculous osteomyelitis, though rare in developed countries, continues to be a common condition in under-developed and developing countries of the world. The tubercle bacilli, *M. tuberculosis*, reach the bone marrow and synovium most commonly by haematogenous dissemination from infection elsewhere, usually the lungs, and infrequently by direct extension from the pulmonary or gastrointestinal tuberculosis (page 125). The disease affects adolescents and young adults more often. Most frequently involved are the spine and bones of extremities.

PATHOLOGIC CHANGES. The bone lesions in tuberculosis have the same general histological appearance as in tuberculosis elsewhere and consist of central caseation necrosis surrounded by tuberculous granulation tissue. The tuberculous lesions appear as a focus of bone destruction and replacement of the affected tissue by caseous material and formation of multiple discharging sinuses through the soft tissues and skin. Involvement of joint spaces and intervertebral disc are frequent. Tuberculosis of the spine, *Pott's disease*, often commences in the vertebral body and may be associated with compression fractures and destruction of intervertebral discs, producing permanent damage and paraplegia. Extension of caseous material along with pus from the lumbar vertebrae to the sheaths of psoas muscle produces *psoas abscess* or *lumbar cold abscess* (Fig. 32.4,B). The cold abscess may burst through the skin and form sinus. Long-standing cases may develop systemic amyloidosis.

FRACTURE HEALING

Fracture of the bone initiates a series of tissue changes which eventually lead to restoration of normal structure and function of the affected bone. Fracture of a bone is commonly associated with injury to the soft tissues. The various types of fractures and their mechanism of healing are discussed alongwith healing of specialised tissues in Chapter 12 (page 141).

OSTEOPOROSIS

Osteoporosis or osteopenia is a common clinical syndrome involving multiple bones in which there is quantitative reduction of bone tissue mass but the bone tissue mass is otherwise normal. This reduction in bone mass results in fragile skeleton which is associated with increased risk of fractures and consequent pain and

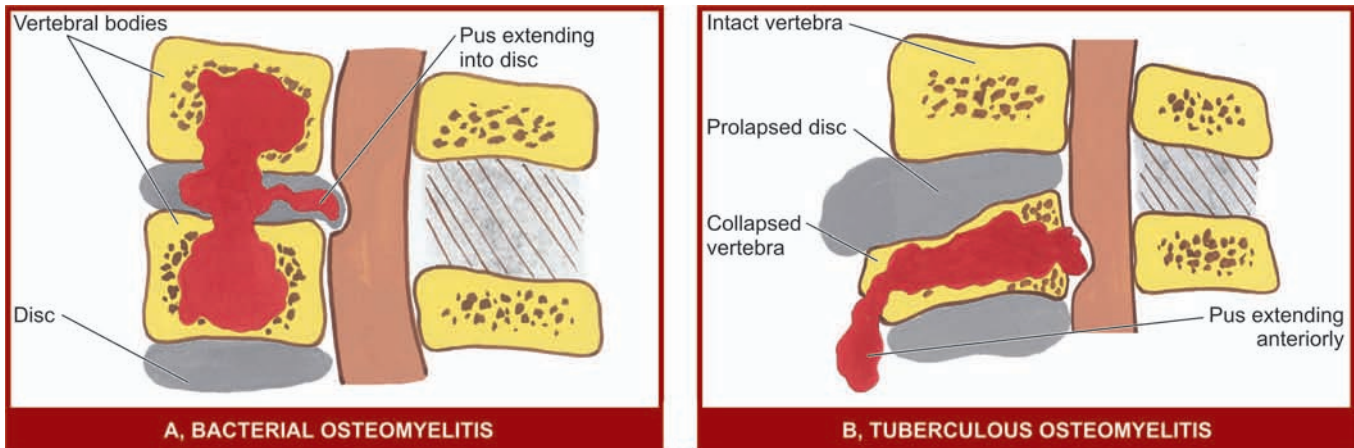


FIGURE 32.4

Osteomyelitis of the vertebral body.

deformity. The condition is particularly common in elderly people and more frequent in postmenopausal women. The condition may remain asymptomatic or may cause only backache. However, more extensive involvement is associated with fractures, particularly of distal radius, femoral neck and vertebral bodies. Osteoporosis may be difficult to distinguish radiologically from other osteopenias such as osteomalacia, osteogenesis imperfecta, osteitis fibrosa of hyperparathyroidism, renal osteodystrophy and multiple myeloma. Radiologic evidence becomes apparent only after more than 30% of bone mass has been lost. Levels of serum calcium, inorganic phosphorus and alkaline phosphatase are usually within normal limits.

PATHOGENESIS. Osteoporosis is conventionally classified into 2 major groups: primary and secondary.

■ **Primary osteoporosis** results primarily from osteopenia without an underlying disease or medication. Primary osteoporosis is further subdivided into 2 types: *idiopathic type* found in the young and juveniles and is less frequent, and *involutional type* seen in postmenopausal women and aging individuals and is more common. The exact mechanism of primary osteoporosis is not known but there is a suggestion that it is the result of an excessive osteoclastic resorption and slow bone formation. A number of risk factors have been attributed to cause this imbalance between bone resorption and bone formation. These include the following:

1. *Genetic factors*—more marked in whites and Asians than blacks.
2. *Sex*—more frequent in females than in males.
3. *Reduced physical activity*—as in old age.

4. *Deficiency of sex hormones*—oestrogen deficiency in women as in postmenopausal osteoporosis and androgen deficiency in men.

5. *Combined deficiency of calcitonin and oestrogen.*

6. *Hyperparathyroidism.*

7. *Deficiency of vitamin D.*

8. *Local factors*—which may stimulate osteoclastic resorption or slow osteoblastic bone formation.

■ **Secondary osteoporosis** is attributed to a number of factors and conditions (e.g. immobilisation, chronic anaemia, acromegaly, hepatic disease, hyperparathyroidism, hypogonadism, thyrotoxicosis and starvation), or as an effect of medication (e.g. hypercortisone, administration of anticonvulsant drugs and large dose of heparin).

PATHOLOGIC CHANGES. Except disuse or immobilisation osteoporosis which is localised to the affected limb, other forms of osteoporosis have systemic skeletal distribution. Most commonly encountered osteoporotic fractures are: vertebral crush fracture, femoral neck fracture and wrist fracture. There is enlargement of the medullary cavity and thinning of the cortex.

Microscopically, osteoporosis may be active or inactive type.

■ **Active osteoporosis** is characterised by increased bone resorption and formation i.e. *accelerated turnover*. There is increase in the number of osteoclasts with increased resorptive surface as well as increased quantity of osteoid with increased osteoblastic surfaces. The width of osteoid seams is normal.

■ **Inactive osteoporosis** has the features of minimal bone formation and reduced resorptive activity i.e. *reduced turnover*. Histological changes of inactive osteoporosis include decreased number of osteoclasts with decreased resorptive surfaces, and normal or reduced amount of osteoid with decreased osteoblastic surface. The width of osteoid seams is usually reduced or may be normal.

OSTEITIS FIBROSA CYSTICA

Hyperparathyroidism of primary or secondary type results in oversecretion of parathyroid hormone which causes increased osteoclastic resorption of the bone. Here, skeletal manifestations of hyperparathyroidism are considered. Severe and prolonged hyperparathyroidism results in osteitis fibrosa cystica. The lesion is generally induced as a manifestation of primary hyperparathyroidism, and less frequently, as a result of secondary hyperparathyroidism such as in chronic renal failure (renal osteodystrophy).

The clinical manifestations of bone disease in hyperparathyroidism are its susceptibility to fracture, skeletal deformities, joint pains and dysfunctions as a result of deranged weight bearing. The bony changes may disappear after cure of primary hyperparathyroidism such as removal of functioning adenoma. The chief biochemical abnormality of excessive parathyroid hormone is hypercalcaemia, hypophosphataemia and hypercalciuria.

PATHOLOGIC CHANGES. The bone lesions of primary hyperparathyroidism affect the long bones more severely and may range from minor degree of generalised bone rarefaction to prominent areas of bone destruction with cyst formation or brown tumours.

Grossly, there are focal areas of erosion of cortical bone and loss of lamina dura at the roots of teeth.

Microscopically, the following sequential changes appear over a period of time:

■ Earliest change is *demineralisation* and *increased bone resorption* beginning at the subperiosteal and endosteal surface of the cortex and then spreading to the trabecular bone.

■ There is replacement of bone and bone-marrow by fibrosis coupled with increased number of bizarre osteoclasts at the surfaces of moth-eaten trabeculae and cortex (*osteitis fibrosa*).

■ As a result of increased resorption, microfractures and microhaemorrhages occur in the marrow cavity leading to development of cysts (*osteitis fibrosa cystica*).

■ Haemosiderin-laden macrophages and multinucleate giant cells appear at the areas of haemorrhages producing an appearance termed as '*brown tumour*' or '*reparative giant cell granuloma of hyperparathyroidism*' requiring differentiation from giant cell tumour or osteoclastoma (page 430). However, the so-called brown tumours, unlike osteoclastoma, are not true tumours but instead regress or disappear on surgical removal of hyperplastic or adenomatous parathyroid tissue.

RENAL OSTEODYSTROPHY (METABOLIC BONE DISEASE)

Renal osteodystrophy is a loosely used term that encompasses a number of skeletal abnormalities appearing in cases of chronic renal failure and in patients treated by dialysis for several years. Renal osteodystrophy is more common in children than in adults. Clinical symptoms of bone disease in advanced renal failure appear in less than 10% of patients but radiologic and histologic changes are observed in fairly large proportion of cases.

PATHOGENESIS. Renal osteodystrophy involves two main events: *hyperphosphataemia* and *hypocalcaemia* which, in turn, leads to parathormone elaboration and resultant osteoclastic activity and major lesions of renal osteodystrophy—osteomalacia (rickets in children), secondary hyperparathyroidism, osteitis fibrosa cystica, osteosclerosis and metastatic calcification.

The mechanisms underlying renal osteodystrophy are schematically illustrated in Fig. 32.5 and briefly outlined below:

1. Hyperphosphataemia: In CRF, there is impaired renal excretion of phosphate, causing phosphate retention and hyperphosphataemia. Hyperphosphataemia, in turn, causes hypocalcaemia which is responsible for secondary hyperparathyroidism.

2. Hypocalcaemia: Hypocalcaemia may also result from the following:

■ Due to renal dysfunction, there is decreased conversion of vitamin D metabolite 25(OH) cholecalciferol to its active form 1,25 (OH)₂ cholecalciferol.

■ Reduced intestinal absorption of calcium.

3. Parathormone secretion: Hypocalcaemia stimulates secretion of parathormone, eventually leading to secondary hyperparathyroidism which, in turn, causes increased osteoclastic activity.

4. Metabolic acidosis: As a result of decreased renal function, acidosis sets in which may cause osteoporosis and bone decalcification.

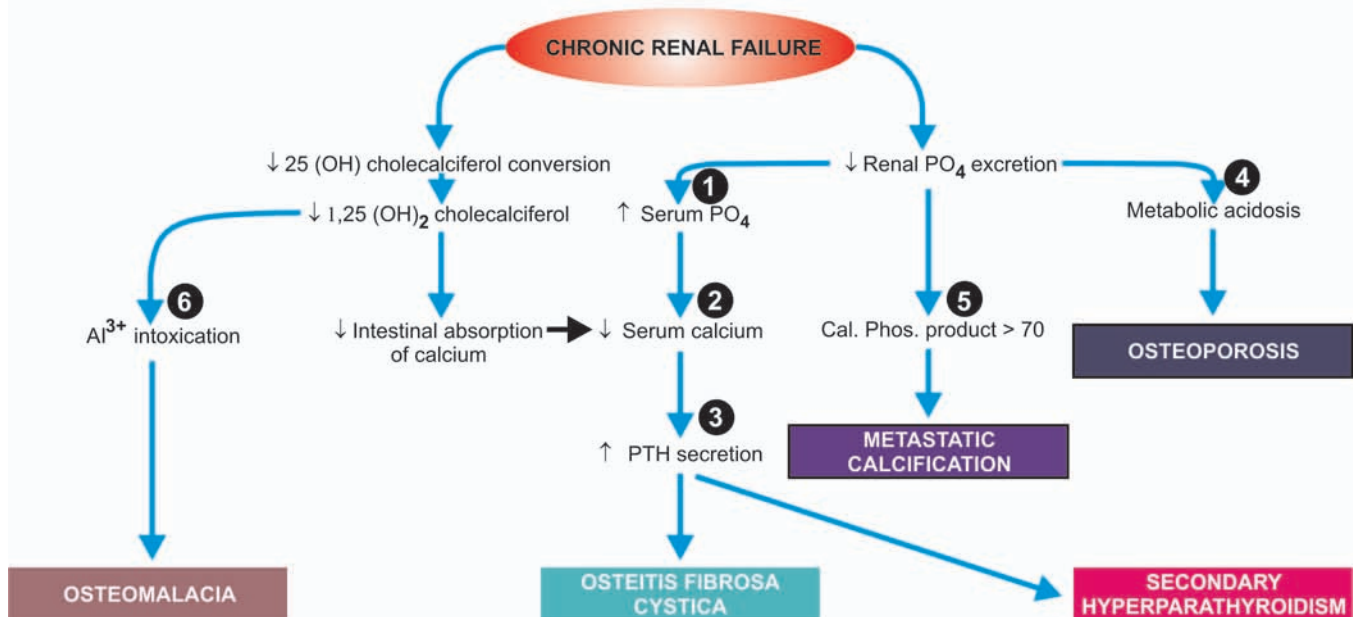


FIGURE 32.5

Pathogenesis of renal osteodystrophy in chronic renal failure. Circled serial numbers in the graphic representation correspond to the sequence described in the text on pathogenesis.

5. Calcium phosphorus product > 70: When the product of biochemical value of calcium and phosphate is higher than 70, metastatic calcification may occur at extraosseous sites.

6. Dialysis-related metabolic bone disease: Long-term dialysis employing use of aluminium-containing dialysate is currently considered to be a major cause of metabolic bone lesions. Aluminium interferes with deposition of calcium hydroxyapatite in bone and results in osteomalacia, secondary hyperparathyroidism and osteitis fibrosa cystica. In addition, accumulation of β_2 -microglobulin amyloid in such cases causes dialysis-related amyloidosis (page 57).

PATHOLOGIC CHANGES. The following skeletal lesions can be identified in renal osteodystrophy:

1. *Mixed osteomalacia-osteitis fibrosa* is the most common manifestation of renal osteodystrophy resulting from disordered vitamin D metabolism and secondary hyperparathyroidism.
2. *Pure osteitis fibrosa* results from metabolic complications of secondary hyperparathyroidism.
3. *Pure osteomalacia* of renal osteodystrophy is attributed to aluminium toxicity.
4. *Renal rickets* resembling the changes seen in children with nutritional rickets with widened osteoid seams may occur (page 88).

5. *Osteosclerosis* is characterised by enhanced bone density in the upper and lower margins of vertebrae.
6. *Metastatic calcification* is seen at extraosseous sites such as in medium-sized blood vessels, periarticular tissues, myocardium, eyes, lungs and gastric mucosa (page 43).

SKELETAL FLUOROSIS

Fluorosis of bones occurs due to high sodium fluoride content in soil and water consumed by people in some geographic areas and is termed endemic fluorosis. Such endemic regions exist in some tropical and subtropical areas; in India it exists in parts of Punjab and Andhra Pradesh. The condition affects farmers who consume drinking water from wells. Non-endemic fluorosis results from occupational exposure in manufacturing industries of aluminium, magnesium, and superphosphate.

PATHOGENESIS. In fluorosis, fluoride replaces calcium as the mineral in the bone and gets deposited without any regulatory control. This results in heavily mineralised bones which are thicker and denser but are otherwise weak and deformed (just as in osteopetrosis). In addition, there are also deposits of fluoride in soft tissues, particularly as nodules in the interosseous membrane. The patient develops skeletal deformities and mottling of teeth.

PATHOLOGIC CHANGES. *Grossly*, the long bones and vertebrae develop nodular swellings present both inside the bones and on the surface.

Microscopically, these nodules are composed of heavily mineralised irregular osteoid admixed with fluoride which requires confirmation chemically.

PAGET'S DISEASE OF BONE (OSTEITIS DEFORMANS)

Paget's disease of bone or osteitis deformans was first described by Sir James Paget in 1877. Paget's disease of bone is an osteolytic and osteosclerotic bone disease of uncertain etiology involving one (monostotic) or more bones (polyostotic). The condition affects predominantly males over the age of 50 years. Though the etiology remains obscure, recently there has been a suggestion that osteitis deformans is a form of slow-virus infection by paramyxovirus detected in osteoclasts. Autosomal dominant inheritance has also been proposed on the basis of observation of disease in families.

Clinically, the *monostotic form* of the disease may remain asymptomatic and the lesion is discovered incidentally or on radiologic examination. *Polyostotic form*, however, is more widespread and may produce pain, fractures, skeletal deformities, and occasionally, sarcomatous transformation. Typically, there is marked elevation of serum alkaline phosphatase and normal to high serum calcium level.

PATHOLOGIC CHANGES. Monostotic Paget's disease involves most frequently: tibia, pelvis, femur, skull and vertebra, while the order of involvement in polyostotic Paget's disease is: vertebrae, pelvis, femur, skull, sacrum and tibia. Three sequential stages are identified in Paget's disease:

- 1. Initial osteolytic stage:** This stage is characterised by areas of osteoclastic resorption produced by increased number of large osteoclasts.
- 2. Mixed osteolytic-osteoblastic stage:** In this stage, there is imbalance between osteoblastic laying down of new bone and osteoclastic resorption so that mineralisation of the newly-laid matrix lags behind, resulting in development of characteristic *mosaic pattern* of osteoid seams or cement lines. The narrow space between the trabeculae and cortex is filled with collagen which gradually becomes less vascular.
- 3. Quiescent osteosclerotic stage:** After many years, excessive bone formation results so that the bone becomes more compact and dense producing osteosclerosis. However, newly-formed bone is poorly mineralised, soft and susceptible to fractures. Radio-

logically, this stage produces characteristic *cotton-wool appearance* of the affected bone.

TUMOUR-LIKE LESIONS OF BONE

In the context of bones, several non-neoplastic conditions resemble true neoplasms and have to be distinguished from them clinically, radiologically and morphologically. Table 32.1 gives a list of such tumour-like lesions. A few common conditions are described below.

Fibrous Dysplasia

Fibrous dysplasia is not an uncommon tumour-like lesion of the bone. It is a benign condition, possibly of developmental origin, characterised by the presence of localised area of replacement of bone by fibrous connective tissue with a characteristic whorled pattern and containing trabeculae of woven bone. Radiologically, the typical focus of fibrous dysplasia has well-demarcated ground-glass appearance.

Three types of fibrous dysplasia are distinguished—monostotic, polyostotic, and Albright syndrome.

■ **Monostotic fibrous dysplasia.** Monostotic fibrous dysplasia affects a solitary bone and is the most common type, comprising about 70% of all cases. The condition affects either sex and most patients are between 20 and 30 years of age. The bones most often affected, in descending order of frequency, are: ribs, craniofacial bones (especially maxilla), femur, tibia and humerus. The condition generally remains asymptomatic and is discovered incidentally, but infrequently may produce tumour-like enlargement of the affected bone.

■ **Polyostotic fibrous dysplasia.** Polyostotic form of fibrous dysplasia affecting several bones constitutes about 25% of all cases. Both sexes are affected equally but the lesions appear at a relatively earlier age than the monostotic form. Most frequently affected bones are: craniofacial, ribs, vertebrae and long bones of the limbs. Approximately a quarter of cases with polyostotic form have more than half of the skeleton involved by

TABLE 32.1: Classification of Tumour-like Lesions of Bone.

1. Fibrous dysplasia
2. Fibrous cortical defect (metaphyseal fibrous defect, non-ossifying fibroma)
3. Solitary bone cyst (simple or unicameral bone cyst)
4. Aneurysmal bone cyst
5. Ganglion cyst of bone (intraosseous ganglion)
6. Brown tumour of hyperparathyroidism (reparative granuloma)
7. Histiocytosis-X (Langerhans' cell histiocytosis) (page 537)

disease. The lesions may affect one side of the body or may be distributed segmentally in a limb. Spontaneous fractures and skeletal deformities occur in childhood polyostotic form of the disease.

■ **Albright syndrome.** Also called McCune-Albright syndrome, this is a form of polyostotic fibrous dysplasia associated with endocrine dysfunctions and accounts for less than 5% of all cases. Unlike monostotic and polyostotic varieties, Albright syndrome is more common in females. The syndrome is characterised by polyostotic bone lesions, skin pigmentation (*cafe-au-lait* macular spots) and sexual precocity, and infrequently other endocrinopathies.

PATHOLOGIC CHANGES. All forms of fibrous dysplasia have an identical pathologic appearance.

Grossly, the lesions appear as sharply-demarcated, localised defects measuring 2-5 cm in diameter, present within the cancellous bone, having thin and smooth overlying cortex. The epiphyseal cartilages are generally spared in the monostotic form but involved in the polyostotic form of disease. Cut section of the lesion shows replacement of normal cancellous bone of the marrow cavity by gritty, grey-pink, rubbery soft tissue which may have areas of haemorrhages, myxoid change and cyst formation.

Microscopically, the lesions of fibrous dysplasia have characteristic benign-looking fibroblastic tissue arranged in a loose, whorled pattern in which there are irregular and curved trabeculae of woven (non-lamellar) bone. There may be numerous osteoclasts in relation to bony trabeculae. Rarely, malignant change may occur in fibrous dysplasia, most often an osteogenic sarcoma.

Fibrous Cortical Defect (Metaphyseal Fibrous Defect, Non-ossifying Fibroma)

Fibrous cortical defect or metaphyseal fibrous defect is a rather common benign tumour-like lesion occurring in the metaphyseal cortex of long bones in children. Most commonly involved bones are upper or lower end of tibia or lower end of femur. The lesion is generally solitary but rarely there may be multiple and bilaterally symmetrical defects. Radiologically, the lesion is eccentrically located in the metaphysis and has a sharply-delimited border. The pathogenesis of fibrous cortical defect is unknown. Possibly, it arises as a result of some developmental defect at the epiphyseal plate, or could be a tumour of histiocytic origin because of close resemblance to fibrohistiocytic tumours (page 289).

Clinically, fibrous cortical defect causes no symptoms and is usually discovered accidentally when X-ray of the region is done for some other reason.

PATHOLOGIC CHANGES. **Grossly,** the lesion is generally small, less than 4 cm in diameter, granular and brown. Larger lesion (5-10 cm) occurring usually in response to trauma is referred to as non-ossifying fibroma.

Microscopically, fibrous cortical defect consists of cellular masses of fibrous tissue showing storiform pattern. There are numerous multinucleate osteoclast-like giant cells, haemosiderin-laden macrophages and foamy cells. That is why the lesion is also termed histiocytic xanthogranuloma or fibrous xanthoma of bone.

Solitary (Simple, Unicameral) Bone Cyst

Solitary, simple or unicameral bone cyst is a benign condition occurring in children and adolescents, most frequently located in the metaphyses at the upper end of humerus and femur. The cyst expands the bone causing thinning of the overlying cortex. The pathogenesis is unknown. Clinically, solitary bone cyst may remain asymptomatic or may cause pain and fracture.

PATHOLOGIC CHANGES. **Grossly,** the simple cyst of the bone is generally unilocular with smooth inner surface. The cavity is filled with clear fluid.

Microscopically, the cyst wall consists of thin collagenous tissue having scattered osteoclast giant cells and newly formed reactive bony trabeculae. Fracture alters the appearance and produces sanguineous fluid in the cavity, and haemorrhages, haemosiderin deposits and macrophages in the cyst wall.

Aneurysmal Bone Cyst

Aneurysmal bone cyst, true to its name, is an expanding osteolytic lesion filled with blood (*aneurysm* = dilatation, distension). The condition is seen more commonly in young patients under 30 years of age. Most frequently involved bones are shafts of metaphyses of long bones or the vertebral column. The radiographic appearance shows characteristic ballooned-out expansile lesion underneath the periosteum. The pathogenesis is not clear but it has been suggested by some authors that the condition probably arises from persistent local alteration in haemodynamics. Clinically, the aneurysmal bone cyst may enlarge over a period of years and produce pain, tenderness and pathologic fracture.

PATHOLOGIC CHANGES. *Grossly*, the lesion consists of a large haemorrhagic mass covered over by thinned out reactive bone (Fig. 32.6,A).

Microscopically, the cyst consists of blood-filled aneurysmal spaces of variable size, some of which are endothelium-lined. The spaces are separated by connective tissue septa containing osteoid tissue, numerous osteoclast-like multinucleate giant cells and trabeculae of bone (Fig. 32.6,B). The condition has to be distinguished histologically from giant cell tumour or osteoclastoma (page 430) and telangiectatic osteosarcoma (page 427).

Ganglion Cyst of Bone

Ganglion cyst of bone or intraosseous ganglion is a benign condition occurring in middle-aged patients. It usually involves lower end of the tibia or humerus. Radiographically, it appears as well-defined osteolytic area within the bone with surrounding zone of sclerosis close to the joint space.

PATHOLOGIC CHANGES. *Grossly*, the ganglion cyst of bone is often multiloculated and is mucus-filled.

Microscopically, it resembles the usual soft tissue ganglion but lacks a synovial lining.

BONE TUMOURS

Bone tumours are comparatively infrequent but they are clinically quite significant since some of them are

highly malignant. Bone tumours may be primary or metastatic. Since histogenesis of some bone tumours is obscure, the WHO has recommended a widely accepted classification of primary bone tumours based on both histogenesis and histologic criteria. Table 32.2 lists the various types of bone tumours arising from different tissue components—osseous and non-osseous, indigenous to the bone. However, in the discussion below, only osseous bone tumours are considered, while non-osseous bone tumours are described elsewhere in the book. The anatomic origin of common primary bone tumours is illustrated in Fig. 32.7.

It may be mentioned here that the diagnosis of any bone lesion is established by a combination of clinical, radiological and pathological examination, supplemented by biochemical and haematological investigations wherever necessary. These include: serum levels of calcium, phosphorus, alkaline phosphatase and acid phosphatase. Specific investigations like plasma and urinary proteins and bone marrow examination in case of myeloma, urinary catecholamines in metastatic neuroblastoma and haematologic profile in lymphoma and leukaemic involvement of bone, are of considerable help.

BONE-FORMING (OSTEOBLASTIC) TUMOURS

Bone-forming or osteoblastic group of bone tumours is characterised by the common property of synthesis of osteoid or bone, or both, directly by the tumour cells (osteogenesis). Formation of reactive bone and endo-

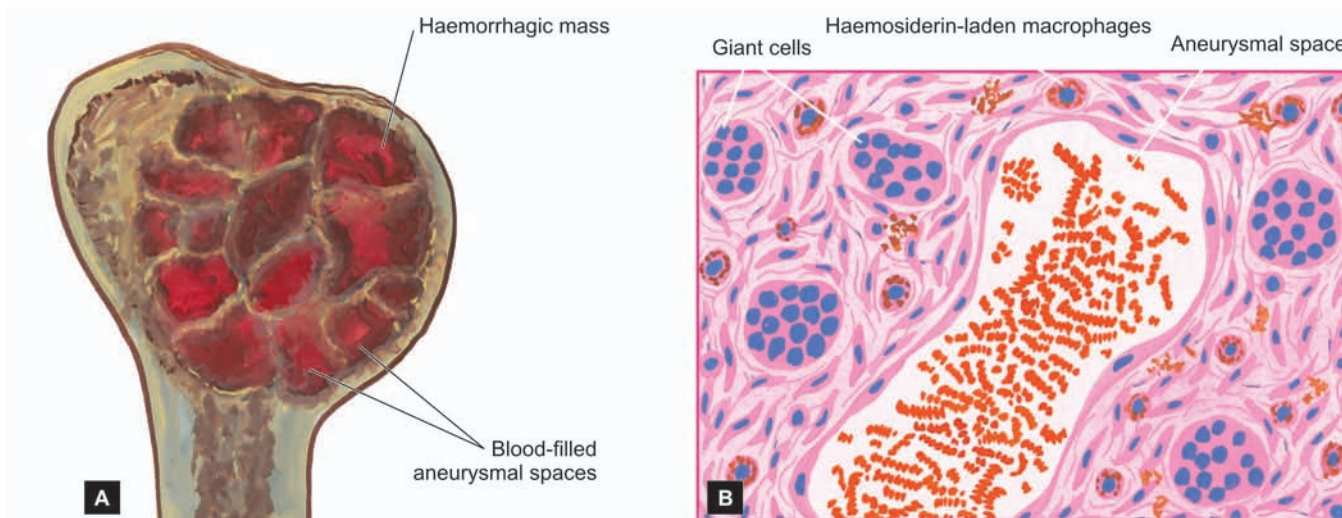


FIGURE 32.6

Aneurysmal bone cyst. A, Characteristic gross appearance of ballooned-out expansile lesion. B, Histologic hallmark of lesion is presence of aneurysmal spaces filled with blood, partly lined by endothelium and separated by connective tissue septa containing osteoclast-like giant cells along the wall of vascular spaces.

TABLE 32.2: Classification of Primary Bone Tumours.

HISTOLOGIC DERIVATION	BENIGN	MALIGNANT
A. OSSEOUS TUMOURS		
I. Bone-forming (osteogenic, osteoblastic) tumours	Osteoma (40-50 yrs) Osteoid osteoma (20-30 yrs) Osteoblastoma (20-30 yrs)	Osteosarcoma (10-20 yrs) Parosteal (juxtacortical) osteosarcoma (50-60 yrs)
II. Cartilage-forming (chondrogenic) tumours	Enchondroma (20-50 yrs) Osteochondroma (20-50 yrs) (Osteocartilaginous exostosis) Chondroblastoma (10-20 yrs) Chondromyxoid fibroma (20-30 yrs)	Chondrosarcoma (40-60 yrs)
III. Haematopoietic (marrow) tumours	—	Myeloma (50-60 yrs) Lymphoplasmacytic lymphoma (50-60 yrs)
IV. Unknown	Giant cell tumour (20-40 yrs) (osteoclastoma)	Malignant giant cell tumour (30-50 yrs) Ewing's sarcoma (5-20 yrs) Adamantinoma of long bones (page 349)
V. Notochordal tumour	—	Chordoma (40-50 yrs)
B. NON-OSSEOUS TUMOURS		
I. Vascular tumours	Haemangioma	Haemangioendothelioma Haemangiopericytoma Angiosarcoma
II. Fibrogenic tumours	Non-ossifying fibroma (metaphyseal fibrous defect)	Fibrosarcoma
III. Neurogenic tumours	Neurilemmoma and neurofibroma	Neurofibrosarcoma
IV. Lipogenic tumours	Lipoma	Liposarcoma
V. Histiocytic tumours	Fibrous histiocytoma	Malignant fibrous histiocytoma

Figures in brackets indicate common age of occurrence.

chondral ossification should not be construed as osteogenesis. Benign bone-forming tumours include: osteoma, osteoid osteoma and osteoblastoma, while the malignant counterpart is osteosarcoma (osteogenic sarcoma).

Osteoma

An osteoma is a rare benign, slow-growing lesion, regarded by some as a hamartoma rather than a true neoplasm. Similar lesions may occur following trauma, subperiosteal haematoma or local inflammation. Osteoma is almost exclusively restricted to the skull and facial bones. It may grow into paranasal sinuses or protrude into the orbit. An osteoma may form a component of Gardner's syndrome. Radiologic appearance is of a dense ivory-like bony mass.

PATHOLOGIC CHANGES. The lesion is composed of well-differentiated mature lamellar bony trabeculae separated by fibrovascular tissue.

Osteoid Osteoma and Osteoblastoma

Osteoid osteoma and osteoblastoma (or giant osteoid osteoma) are closely related benign tumours occurring in children and young adults. Osteoid osteoma is more common than osteoblastoma. There are no clear-cut histologic criteria to distinguish the two. The distinction between them is based on clinical features, size and radiographic appearance.

■ **Osteoid osteoma** is generally small (usually less than 1 cm) and painful tumour, located in the cortex of a long bone. The tumour is clearly demarcated having surrounding zone of reactive bone formation so that radiographically it appears as a small radiolucent central focus or nidus surrounded by dense sclerotic bone.

■ **Osteoblastoma**, on the other hand, is larger in size (usually more than 1 cm), painless, located in the medulla, commonly in the vertebrae, ribs, ilium and long bones, and there is absence of reactive bone formation.

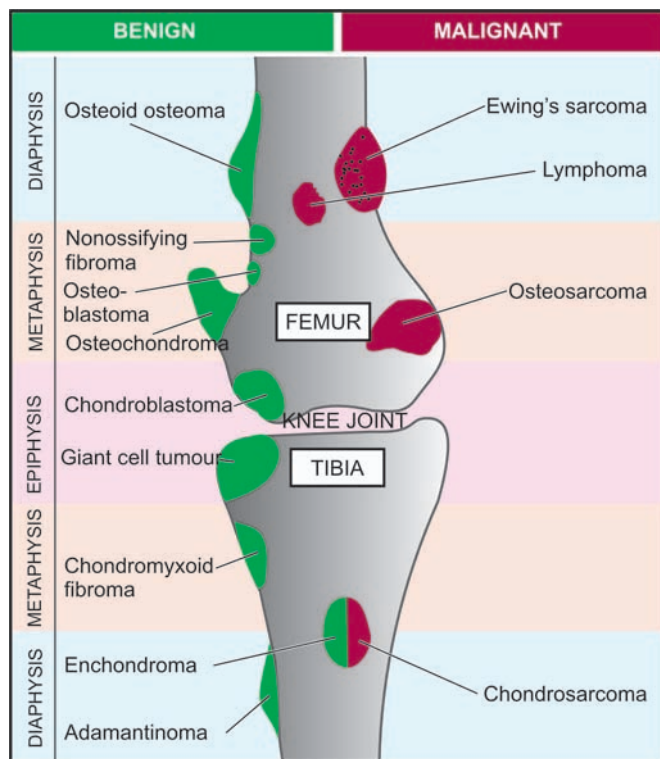


FIGURE 32.7

Anatomic locations of common primary bone tumours.

Microscopically, the distinction between osteoid osteoma and osteoblastoma is not obvious. In either case, the lesion consists of trabeculae of osteoid, rimmed by osteoblasts and separated by highly vascularised connective tissue stroma. Later, some of the trabeculae are mineralised and calcified.

Osteosarcoma

Osteosarcoma or osteogenic sarcoma is the most common primary malignant tumour of the bone. The tumour is characterised by formation of *osteoid or bone, or both, directly by sarcoma cells*. The tumour is thought to arise from primitive osteoblast-forming mesenchyme. Depending upon their locations within the bone, osteosarcomas are classified into 2 main categories: *medullary* (central) and *parosteal* (juxtacortical). The latter is an uncommon variety with a better prognosis and is described separately.

Medullary or central osteosarcoma is the more common type and is generally referred to as 'osteosarcoma' if not specified. The tumour occurs in young patients between the age of 10 and 20 years. Males are affected more frequently than females. The tumour arises in the metaphysis of long bones. Most common

sites, in descending order of frequency, are: the lower end of femur and upper end of tibia (i.e. around knee joint about 60%); the upper end of humerus (10%); pelvis and the upper end of femur (i.e. around hip joint about 15%); and less often in jaw bones, vertebrae and skull. Rarely an osteosarcoma may occur in extraskeletal soft tissues.

Based upon the pathogenesis, osteosarcoma is divided into 2 types: primary and secondary.

■ **Primary osteosarcoma** is more common and occurs in the absence of any known underlying disease. Its etiology is unknown but there is evidence linking this form of osteosarcoma with genetic factors (e.g. hereditary mutation of chromosome 13 in common with retinoblastoma locus), period of active bone growth (occurrence of the tumour in younger age), and certain environmental influences (e.g. radiation and an oncogenic virus). Cases of hereditary retinoblastoma have a very high prevalence risk of development of osteosarcoma.

Many sporadic osteosarcomas show mutation in *TP53* tumour suppressor gene; some have over-expression of *MDM2* gene. Patients of hereditary retinoblastoma are predisposed to develop osteosarcoma implicating *RB* gene in their pathogenesis.

■ **Secondary osteosarcoma**, on the other hand, develops following pre-existing bone disease e.g. Paget's disease of bone, fibrous dysplasia, multiple osteochondromas, chronic osteomyelitis, infarcts and fractures of bone. The tumour has a more aggressive behaviour than the primary osteosarcoma.

Medullary osteosarcoma is a highly malignant tumour. The tumour arises centrally in the metaphysis, extends longitudinally for variable distance into the medullary cavity, expands laterally on either side breaking through the cortex and lifting the periosteum. If the periosteum is breached the tumour grows relentlessly into the surrounding soft tissues. The only tissue which is able to stop its spread, *albeit* temporarily, is the cartilage of epiphyseal plate. The radiographic appearance is quite distinctive: characteristic '*sunburst pattern*' due to osteogenesis within the tumour and presence of *Codman's triangle* formed at the angle between the elevated periosteum and underlying surface of the cortex (Fig. 32.8,A).

Clinically, the usual osteosarcoma presents with pain, tenderness and an obvious swelling of affected extremity. Serum alkaline phosphatase level is generally raised but calcium and phosphorus levels are normal. The tumour metastasises rapidly and widely to distant sites by haematogenous route and disseminates commonly to the lungs, other bones, brain and various other sites.

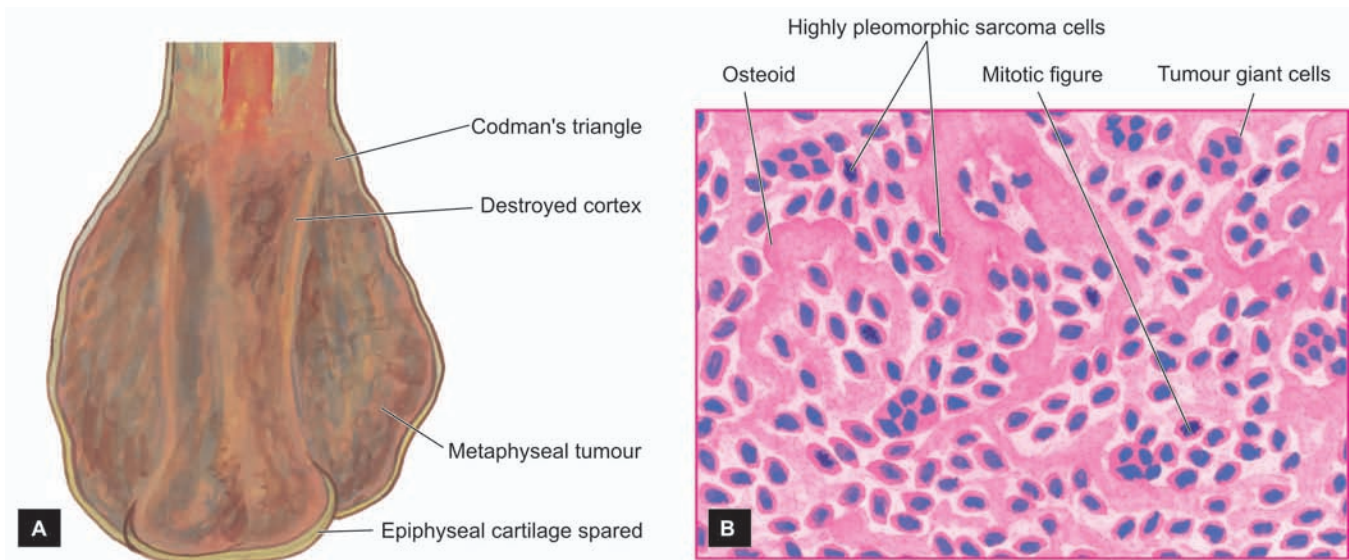


FIGURE 32.8

Osteosarcoma. A, Typical macroscopic appearance of tumour in the lower metaphysis of femur. B, Hallmarks of microscopic picture of the usual osteosarcoma are the sarcoma cells characterised by variation in size and shape of tumour cells, bizarre mitosis and multinucleate tumour giant cells, and osteogenesis i.e. production of osteoid matrix and bone directly by the tumour cells.

PATHOLOGIC CHANGES. *Grossly*, the tumour appears as a grey-white, bulky mass at the metaphyseal end of a long bone of the extremity. The articular end of the bone is generally uninvolved in initial stage. Codman's triangle, though identified radiologically, may be obvious on macroscopic examination (Fig. 32.8,A). Cut surface of the tumour is grey-white with areas of haemorrhages and necrotic bone. Tumours which form abundance of osteoid, bone and cartilage may have hard, gritty and mucoid areas.

Microscopically, osteosarcoma shows considerable variation in pattern from case to case and even within a tumour from one area to the other. However, the following two features characterise all osteosarcomas (Fig. 32.8,B) (**COLOUR PLATE XII: CL 47**):

1. **Sarcoma cells:** The tumour cells of osteosarcomas are undifferentiated mesenchymal stromal cells which show marked pleomorphism and polymorphism i.e. variation in size as well as shape. The tumour cells may have various shapes such as spindled, round, oval and polygonal and bizarre tumour giant cells. The tumour cells have variable size and show hyperchromatism and atypical mitoses.

2. **Osteogenesis:** The anaplastic sarcoma cells form osteoid matrix and bone directly which are found

interspersed in the areas of tumour cells. In addition to osteoid and bone, the tumour cells may produce cartilage, fibrous tissue or myxoid tissue.

A variant of the usual osteosarcoma is *telangiectatic osteosarcoma* in which the tumour has large, cavernous, dilated vascular channels.

Parosteal (Juxtacortical) Osteosarcoma

Parosteal or juxtacortical osteosarcoma is an uncommon form of osteosarcoma having its origin from the external surface of the bone (*parosteal* or *juxtacortical* means outer to cortex). The tumour should be distinguished from the more common medullary osteosarcoma because of its better prognosis and different presentation. The tumour occurs in older age group, has no sex predilection and is slow growing. Its common locations are metaphysis of long bones, most frequently lower end of the femur and upper end of the humerus. X-ray examination usually reveals a dense bony mass attached to the outer cortex of the affected long bone.

PATHOLOGIC CHANGES. *Grossly*, the tumour is lobulated and circumscribed, calcified mass in the subperiosteal location.

Microscopically, though the features which characterise the usual osteosarcoma (sarcomatous stroma and production of neoplastic osteoid and

bone) are present, the tumour shows a high degree of structural differentiation, accounting for distinctly better prognosis in these cases.

CARTILAGE-FORMING (CHONDROBLASTIC) TUMOURS

The tumours which are composed of frank cartilage or derived from cartilage-forming cells are included in this group. It comprises benign lesions like osteocartilaginous exostoses (osteochondromas), enchondroma, chondroblastoma and chondromyxoid fibroma, and a malignant counterpart chondrosarcoma.

Osteocartilaginous Exostoses (Osteochondromas)

Osteocartilaginous exostoses or osteochondromas are the commonest of benign cartilage-forming lesions. Though designated and discussed with neoplasms, exostosis or osteochondroma is not a true tumour but is regarded as a disorder of growth and development. It may occur as a '*solitary sporadic exostosis*' or there may be '*multiple hereditary exostoses*'.

Exostoses arise from metaphyses of long bones as exophytic lesions, most commonly lower femur and upper tibia (i.e. around knee) and upper humerus but may also be found in other bones such as the scapula or ilium. They are discovered most commonly in late childhood or adolescence and are more frequent in males. They may remain asymptomatic and discovered as an incidental radiographic finding or may produce obvious deformity. Both solitary and multiple exostoses may undergo transformation into chondrosarcoma but the risk is much greater with multiple hereditary exostoses.

PATHOLOGIC CHANGES. *Grossly*, osteochondromas have a broad or narrow base (i.e. may be either sessile or pedunculated) which is continuous with the cortical bone. They protrude exophytically as mushroom-shaped, cartilage-capped lesions enclosing well-formed cortical bone and marrow (Fig. 32.9).

Microscopically, they are composed of outer mature cartilage resembling epiphyseal cartilage and the inner mature lamellar bone and bone marrow.

Enchondroma

Enchondroma is the term used for the benign cartilage-forming tumour that develops centrally within the interior of the affected bone, while chondroma refers to the peripheral development of lesion similar to osteochondromas. Enchondromas may occur singly or they may be multiple, forming a non-hereditary disorder

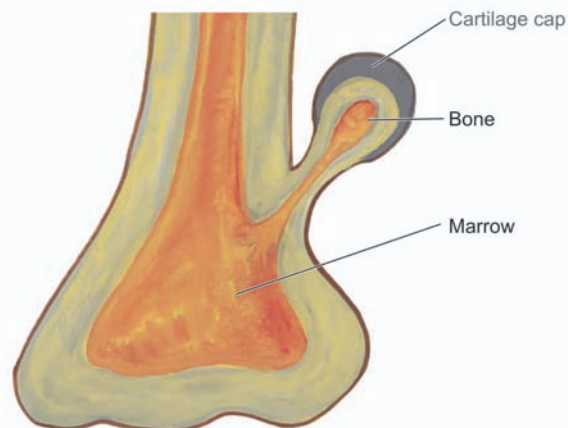


FIGURE 32.9

Characteristic appearance of a solitary osteocartilaginous exostosis (osteochondroma).

called *enchondromatosis* or *Ollier's disease*. The coexistence of multiple enchondromas with multiple soft tissue haemangiomas constitutes a familial syndrome called *Maffucci's syndrome*. Most common locations for enchondromas are short tubular bones of the hands and feet, and less commonly, they involve the ribs or the long bones. They may appear at any age and in either sex. Enchondromas, like osteochondromas, may remain asymptomatic or may cause pain and pathologic fractures. X-ray reveals a radiolucent, lobulated tumour mass with spotty calcification. Malignant transformation of solitary enchondroma is rare but multiple enchondromas may develop into chondrosarcoma.

PATHOLOGIC CHANGES. *Grossly*, the enchondroma is lobulated, bluish-grey, translucent, cartilaginous mass lying within the medullary cavity.

Histologically, the tumour has characteristic lobulated appearance. The lobules are composed of normal adult hyaline cartilage separated by vascularised fibrous stroma. Foci of calcification may be evident within the tumour. Enchondroma is distinguished from chondrosarcoma by the absence of invasion into surrounding tissues and lack of cytologic features of malignancy.

Chondroblastoma

Chondroblastoma is a relatively rare benign tumour arising from the epiphysis of long bones adjacent to the epiphyseal cartilage plate. Most commonly affected bones are upper tibia and lower femur (i.e. about knee) and upper humerus. The tumour usually occurs in

patients under 20 years of age with male preponderance (male-female ratio 2:1). The radiographic appearance is of a sharply-circumscribed, lytic lesion with multiple small foci of calcification. Chondroblastoma may be asymptomatic, or may produce local pain, tenderness and discomfort. The behaviour of the tumour is benign though it may recur locally after curettage.

PATHOLOGIC CHANGES. *Grossly*, chondroblastoma is a well-defined mass, up to 5 cm in diameter, lying in the epiphysis. The tumour is surrounded by thin capsule of dense sclerotic bone. Cut surface reveals a soft chondroid tumour with foci of haemorrhages, necrosis and calcification.

Microscopically, the tumour is highly cellular and is composed of small, round to polygonal mononuclear cells resembling chondroblasts and also contains multinucleate osteoclast-like giant cells. There are small areas of cartilaginous intercellular matrix and focal calcification.

Chondromyxoid Fibroma

Chondromyxoid fibroma is an uncommon benign tumour of cartilaginous origin arising in the metaphysis of long bones. Most common locations are upper end of tibia and lower end of femur i.e. around the knee joint. Majority of tumours appear in 2nd to 3rd decades of life with male preponderance. Radiologically, the tumour appears as a sharply-outlined radiolucent area with foci of calcification and expansion of affected end of the bone. The lesion may be asymptomatic, or may cause pain, swelling and discomfort in the affected joint. The lesions may recur after curettage. Thus, there are many similarities with chondroblastoma.

PATHOLOGIC CHANGES. *Grossly*, chondromyxoid fibroma is sharply-demarcated, grey-white lobulated mass, not exceeding 5 cm in diameter, lying in the metaphysis. The tumour is often surrounded by a layer of dense sclerotic bone. Cut surface of the tumour is soft to firm and lobulated but calcification within the tumour is not as common as with other cartilage-forming tumours.

Microscopically, the tumour has essentially lobulated pattern. The lobules are separated by fibrous tissue and variable number of osteoclast-like giant cells. The lobules themselves are composed of immature cartilage consisting of spindle-shaped or stellate cells with abundant myxoid or chondroid intercellular matrix.

In view of close histogenetic relationship between chondromyxoid fibroma and chondroblastoma, occa-

sional tumours show a combination of histological features of both.

Chondrosarcoma

Chondrosarcoma is a malignant tumour of chondroblasts. In frequency, it is next in frequency to osteosarcoma but is relatively slow-growing and thus has a much better prognosis than that of osteosarcoma. Two types of chondrosarcoma are distinguished: *central* and *peripheral*.

■ **Central chondrosarcoma** is more common and arises within the medullary cavity of diaphysis or metaphysis. This type of chondrosarcoma is generally primary i.e. occurs *de novo*.

■ **Peripheral chondrosarcoma** arises in the cortex or periosteum of metaphysis. It may be primary or secondary occurring on a pre-existing benign cartilaginous tumour such as osteochondroma (osteochondromas), multiple enchondromatosis, and rarely, chondroblastoma.

Both forms of chondrosarcoma usually occur in patients between 3rd and 6th decades of life with slight male preponderance. In contrast to benign cartilaginous tumours, majority of chondrosarcomas are found in the central skeleton (i.e. in the pelvis, ribs and shoulders) and around the knee joint. Radiologic appearance is of hugely expansile and osteolytic growth with foci of calcification. Clinically, the tumour is slow-growing and comes to attention because of pain and gradual enlargement over the years. Lower grades of the tumour recur following surgical removal but higher grades cause metastatic dissemination, commonly to the lungs, liver, kidney and brain.

PATHOLOGIC CHANGES. *Grossly*, chondrosarcoma may vary in size from a few centimeters to extremely large and lobulated masses of firm consistency. Cut section of the tumour shows translucent, bluish-white, gelatinous or myxoid appearance with foci of ossification (Fig. 32.10,A).

Microscopically the hallmarks of chondrosarcoma are invasive character and formation of lobules of anaplastic cartilage cells showing cytologic features of malignancy such as hyperchromatism, pleomorphism, two or more cells in the lacunae and tumour giant cells (Fig. 32.10,B). However, sometimes distinction between a well-differentiated chondrosarcoma and a benign chondroma may be difficult and in such cases location, clinical features and radiological appearance are often helpful.

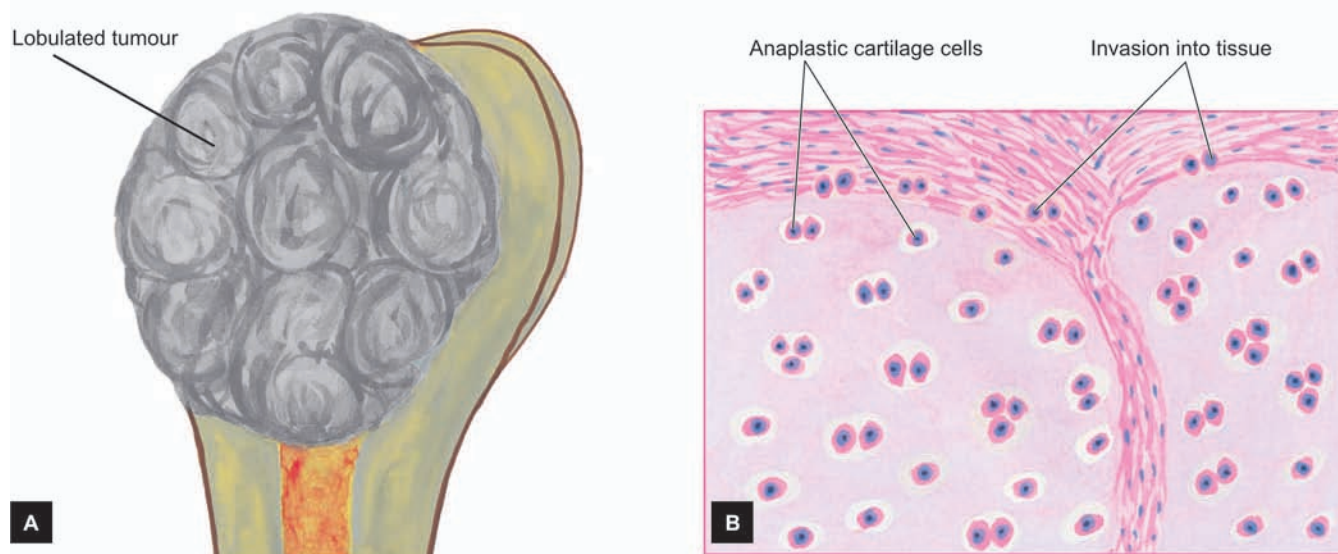


FIGURE 32.10

Chondrosarcoma. A, Gross appearance showing a large lobulated and translucent tumour on cut section (upper end of humerus). B, Histologic features include invasion of the tumour into adjacent soft tissues and cytologic characteristics of malignancy in the tumour cells.

Rare variants of chondrosarcoma are mesenchymal chondrosarcoma, dedifferentiated chondrosarcoma and clear cell chondrosarcoma.

GIANT CELL TUMOUR (OSTEOCLASTOMA)

Giant cell tumour or osteoclastoma is a distinctive neoplasm with uncertain histogenesis and hence classified separately. The tumour arises in the epiphysis of long bones close to the articular cartilage. Most common sites of involvement are lower end of femur and upper end of tibia (i.e. about the knee), lower end of radius and upper end of fibula. Giant cell tumour occurs in patients between 20 and 40 years of age with no sex predilection. Clinical features at presentation include pain, especially on weight-bearing and movement, noticeable swelling and pathological fracture. Radiologically, giant cell tumour appears as a large, lobulated and osteolytic lesion at the end of an expanded long bone with characteristic 'soap bubble' appearance.

PATHOLOGIC CHANGES. *Grossly*, giant cell tumour is eccentrically located in the epiphyseal end of a long bone which is expanded. The tumour is well-circumscribed, dark-tan and covered by a thin shell of subperiosteal bone. Cut surface of the tumour is characteristically haemorrhagic, necrotic, and

honey-combed due to focal areas of cystic degeneration (Fig. 32.11,A).

Microscopically, the hallmark of giant cell tumour is the presence of large number of multinucleate osteoclast-like giant cells which are regularly scattered throughout the stromal mononuclear cells (Fig. 32.11,B) (COLOUR PLATE XII: CL 46):

- **Giant cells** often contain as many as 100 benign nuclei and have many similarities to normal osteoclasts. These cells have very high acid phosphatase activity.

- **Stromal cells** are mononuclear cells and are the real tumour cells and their histologic appearance determines the biologic behaviour of the tumour. Typically, they are uniform, plump, spindle-shaped or round to oval cells with numerous mitotic figures.

- **Other features** of the stroma include its scanty collagen content, rich vascularity, areas of haemorrhages and presence of macrophages.

Giant cell tumour of bone has certain peculiarities which deserve further elaboration. These are: its *cell of origin*, its *differentiation from other giant cell lesions* and its *biologic behaviour*.

- **Cell of origin.** Though designated as giant cell tumour or osteoclastoma, the true tumour cells are round to spindled mononuclear cells and not osteoclast-like giant

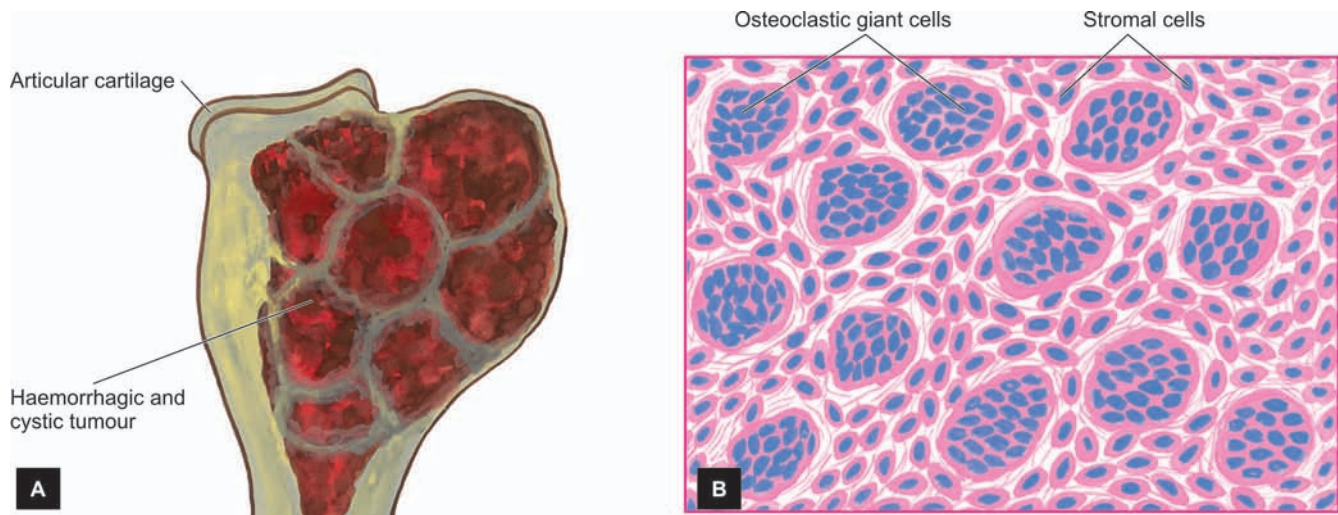


FIGURE 32.11

Giant cell tumour (osteoclastoma). A, Sectioned appearance of tumour in the upper end of tibia showing expansion of epiphysis and haemorrhagic and honey-combed tumour. B, Microscopy reveals osteoclast-like multinucleate giant cells which are regularly distributed among the mononuclear stromal cells.

cells. Histogenesis of tumour cells is uncertain but possibly they are of mesenchymal origin. The available evidence suggests that osteoclasts are perhaps derived from fusion of monocytes and are reactive in nature.

■ **Other giant cell lesions.** This peculiar tumour with above description is named 'giant cell tumour' but giant cells are present in several other benign tumours and tumour-like lesions from which the giant cell tumour is to be distinguished. These *benign giant cell lesions* are: chondroblastoma, brown tumour of hyperparathyroidism, reparative giant cell granuloma, aneurysmal bone cyst, simple bone cyst and metaphyseal fibrous defect (non-ossifying fibroma).

■ **Biologic behaviour.** Giant cell tumours are best described as aggressive lesions. About 40 to 60% of them recur after curettage, sometimes after a few decades of initial resection. Approximately 4% cases result in distant metastases, mainly to lungs. Metastases are histologically benign and there is usually history of repeated curettages and recurrences. Thus attempts at histologic grading of giant cell tumour do not yield satisfactory results. One of the factors considered significant in malignant transformation of this tumour is the role of radiotherapy resulting in development of post-radiation bone sarcoma though primary (*de novo*) malignant or dedifferentiated giant cell tumour may also occur.

EWING'S SARCOMA AND PRIMITIVE NEUROECTODERMAL TUMOUR (PNET)

Ewing's sarcoma is a highly malignant small round cell tumour occurring in patients between the age of 5 and 20 years with predilection for occurrence in females. Since its first description by Ewing in 1921, the histogenesis of this tumour has been a debatable issue. At different times, the possibilities suggested for the cell of origin are endothelial, pericytic, bone marrow, osteoblastic, mesenchymal, and more recently is settled for primitive neuroectodermal cells. Thus, currently Ewing's sarcoma includes:

- i) classic (skeletal) Ewing's sarcoma;
- ii) soft tissue Ewing's sarcoma; and
- iii) primitive neuroectodermal tumour (PNET).

The three are linked together by a common neural origin and by a common cytogenetic translocation abnormality t(11; 22) (q24; q12). This suggests a phenotypic spectrum in these conditions varying from undifferentiated Ewing's sarcoma to PNET positive for rosettes and neural markers (neuron-specific enolase NSE, S-100). However, PNET ultimately has a worse prognosis.

The skeletal Ewing's sarcoma arises in the medullary canal of diaphysis or metaphysis. The common sites are shafts and metaphysis of long bones, particularly femur, tibia, humerus and fibula, although some flat bones such as pelvis and scapula may also be involved.

Clinical features include pain, tenderness and swelling of the affected area accompanied by fever, leucocytosis and elevated ESR. These signs and symptoms may lead to an erroneous clinical diagnosis of osteomyelitis. However, X-ray examination reveals a predominantly osteolytic lesion with patchy subperiosteal reactive bone formation producing characteristic 'onion-skin' radiographic appearance.

PATHOLOGIC CHANGES. *Grossly*, Ewing's sarcoma is typically located in the medullary cavity and produces expansion of the affected diaphysis (shaft) or metaphysis, often extending into the adjacent soft tissues. The tumour tissue is characteristically grey-white, soft and friable (Fig. 32.12,A).

Microscopically, Ewing's tumour is a member of *small round cell tumours* which includes other tumours such as: PNET, neuroblastoma, embryonal rhabdomyosarcoma, lymphoma-leukaemias, and metastatic small cell carcinoma. Ewing's tumour shows the following microscopic characteristics (Fig. 32.12,B):

1. Pattern. The tumour is divided by fibrous septa into irregular lobules of closely-packed tumour cells. These tumour cells are characteristically arranged around capillaries.

2. Tumour cells. The individual tumour cells comprising the lobules are small and uniform resembling

lymphocytes and have ill-defined cytoplasmic outlines, scanty cytoplasm and round nuclei having 'salt and pepper' chromatin and frequent mitoses. Based on these cytological features the tumour is also called *round cell tumour* or *small blue cell tumour*. The cytoplasm contains glycogen that stains with periodic acid-Schiff (PAS) reaction. The tumour cells may be arranged around blood vessels forming *pseudorosettes*.

3. Other features. The tumour is richly vascularised and lacks the intercellular network of reticulin fibres. There may be areas of necrosis and acute inflammatory cell infiltration. Focal areas of reactive bone formation may be present.

Ewing's sarcoma metastasises early by haematogenous route to the lungs, liver, other bones and brain. Involvement of other bones has prompted a suggestion of *multicentric origin* of Ewing's sarcoma. The tumour has to be distinguished microscopically from *other small round cell tumours of bone* such as malignant lymphoma, metastatic neuroblastoma, myeloma, leukaemic deposits and metastatic carcinoma. The prognosis of Ewing's sarcoma used to be dismal (5-year survival rate less than 10%). But currently, use of combined regimen consisting of radiotherapy and systemic chemotherapy has improved the outcome greatly (5-year survival rate 40-75%).

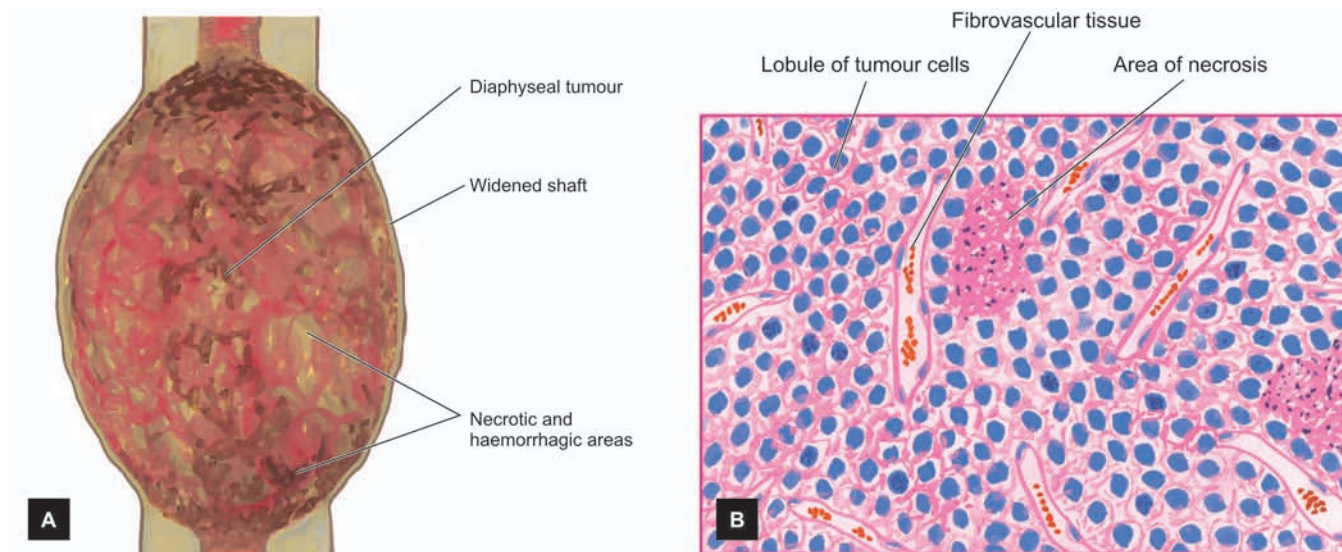


FIGURE 32.12

Ewing's sarcoma. A, Sectioned appearance of a diaphyseal (shaft) tumour in the femur showing characteristic grey-white and soft tumour in expanded medullary cavity. B, Characteristic microscopic features are irregular lobules of uniform small tumour cells with indistinct cytoplasmic outlines which are separated by fibrous tissue septa having rich vascularity. Areas of necrosis and inflammatory infiltrate are also included.

CHORDOMA

Chordoma is a slow-growing malignant tumour arising from remnants of notochord. Notochord is the primitive axial skeleton which subsequently develops into the spine. Normally, remnants of notochord are represented by notochordal or physaliphorous (*physalis* = bubble, *phorous* = bearing) cells present in the nucleus pulposus and a few clumps within the vertebral bodies. Chordomas thus occur in the axial skeleton, particularly sacral and spheno-occipital region, and infrequently in the vertebrae. Chordoma is usually found in patients over the age of 40 years with no sex predilection. Radiographically, the tumour usually appears as an osteolytic lesion. Symptoms of spinal cord compression may be present. The tumour grows slowly and infiltrates adjacent structures but metastases develop rarely. Recurrences after local excision are frequent and the tumour almost invariably proves fatal.

PATHOLOGIC CHANGES. *Grossly*, the tumour is soft, lobulated, translucent and gelatinous with areas of haemorrhages.

Microscopically, chordoma is composed of highly vacuolated physaliphorous cells surrounded by a sea of intercellular mucoid material. Histologic differentiation between chordoma and chondrosarcoma or mucin-secreting carcinoma may sometimes be difficult.

METASTATIC BONE TUMOURS

Metastases to the skeleton are more frequent than the primary bone tumours. Metastatic bone tumours are exceeded in frequency by only 2 other organs—lungs and liver. Most skeletal metastases are derived from haematogenous spread.

Bony metastases of carcinomas predominate over the sarcomas. Some of the common *carcinomas* metastasising to the bones are of: breast, prostate, lung, kidney, stomach, thyroid, cervix, body of uterus, urinary bladder, testis, melanoma and neuroblastoma of adrenal gland. Examples of *sarcomas* which may metastasise to bone are: embryonal and alveolar rhabdomyosarcoma, Ewing's sarcoma and osteosarcoma.

Skeletal metastases may be single or multiple. Most commonly involved bones are: the spine, pelvis, femur, skull, ribs and humerus. Usual radiographic appearance is of an *osteolytic* lesion. *Osteoblastic* bone metastases occur in cancer of the prostate, carcinoid tumour and small cell carcinoma of lung.

Metastatic bone tumours generally reproduce the microscopic picture of primary tumour. Many a times, evidence of skeletal metastases is the first clinical manifestation of an occult primary cancer in the body.



Diseases of Blood and Lympho-reticular Tissues

SECTION CONTENTS

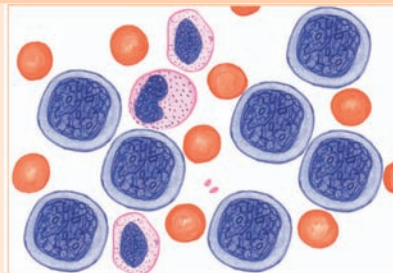
CHAPTER 33: Diseases of Red Blood Cells

CHAPTER 34: Diseases of White Blood Cells

CHAPTER 35: Platelets and Haemorrhagic Diathesis

CHAPTER 36: Blood Groups and Blood Transfusion

CHAPTER 37: Diseases of Lympho-reticular Tissues



33

Diseases of Red Blood Cells

This section on disorders of the haematopoietic system is concerned with diseases of the blood and bone marrow. The account that follows here is arbitrarily divided into the following subsections for the purpose of convenience of discussion:

(1) bone marrow; (2) red blood cells; (3) white blood cells; (4) platelets and haemorrhagic diathesis; (5) blood groups and blood transfusion.

Broad outlines of the treatment of common haematological diseases are also included in the discussion below.

BONE MARROW

The pluripotent stem cells in the bone marrow give rise to two types of multipotent stem cells: *non-lymphoid stem cells* which differentiate in the bone marrow, and *lymphoid stem cells* which differentiate in the bone marrow and then migrate to the lymphoid tissues. The non-lymphoid stem cells form the circulating erythrocytes, granulocytes, monocytes and platelets. Monocytes on entering the tissues form a variety of phagocytic macrophages, both of which together constitute mononuclear-phagocyte system (Chapter 13). Lymphopoietic cells in the marrow undergo differentiation to form B and T lymphocytes of the immune system.

Circulating blood normally contains 3 main types of cells—the red cells (erythrocytes), the white cells (leucocytes) and the platelets (thrombocytes). These blood cells perform their respective physiologic functions: *erythrocytes* are largely concerned with oxygen transport, *leucocytes* play various roles in body defense against infection and tissue injury, while *thrombocytes* are primarily involved in maintaining integrity of blood vessels and in preventing blood loss. The life-span of these cells is variable—neutrophils have the blood life-span of 6-8 hours, followed by platelets with a life-span of 10 days, while the RBCs have the longest life-span of 90-120 days. The rates of production of these blood cells are normally regulated in healthy individuals in such a way so as to match the rate at which they are lost from circulation. Their concentration is normally maintained within well-defined limits unless the balance is disturbed due to some pathologic processes.

HAEMATOPOIESIS

In the human embryo, the *yolk sac* is the main site of haematopoiesis in the first few weeks of gestation. By about 3rd month, however, the *liver and spleen* are the main sites of blood cell formation and continue to do so until about 2 weeks after birth. Haematopoiesis

commences in the *bone marrow* by 4th and 5th month and becomes fully active by 7th and 8th month so that at birth practically all the bones contain active marrow. During normal childhood and adult life, therefore, the marrow is the only source of new blood cells. However, during childhood, there is progressive fatty replacement throughout the long bones so that by adult life the haematopoietic marrow is confined to the central skeleton (vertebrae, sternum, ribs, skull, sacrum and pelvis) and proximal ends of femur, tibia and humerus (Fig. 33.1). Even in these haematopoietic areas, about 50% of the marrow consists of fat. Non-haematopoietic marrow in the adult is, however, capable of reverting to active haematopoiesis in certain pathologic conditions. The spleen and liver can also resume their foetal haematopoietic role in certain pathologic conditions and is called *extramedullary haematopoiesis*.

In the bone marrow, the developing blood cells are situated outside the marrow sinuses, from where on maturation they enter the marrow sinuses, the marrow microcirculation and thence released into circulation.

HAEMATOPOIETIC STEM CELLS

It is widely accepted that blood cells develop from a small population of common multipotent haematopoietic stem cells. The stem cells express a variety of cell surface proteins such as CD34 and adhesion proteins which help the stem cells to "home" to bone marrow when infused. The stem cells have the appearance of small or intermediate-sized lymphocytes and their presence in the marrow can be demonstrated by cell culture techniques by the growth of colony-forming units (CFU) pertaining to different cell lines. The stem cells have the capability of maintaining their progeny by self-replication. The bone marrow provides a suitable environment for growth and development of stem cells. For instance, if haematopoietic stem cells are infused intravenously into a suitably-prepared recipient, they seed the marrow successfully but do not thrive at other sites. This principle forms the basis of bone marrow transplantation performed for various haematologic diseases.

The stem cells, after a series of divisions, differentiate into two types of progenitors—*lymphoid* (immune system) stem cells, and *non-lymphoid or myeloid* (trilineage) stem cells. The former develop into T, B and null lymphocytes while the latter differentiate into 3 types of cell lines—granulocyte-monocyte progenitors (producing neutrophils, eosinophils, basophils and monocytes), erythroid progenitors (producing red cells), and megakaryocytes (as the source of platelets). The develop-

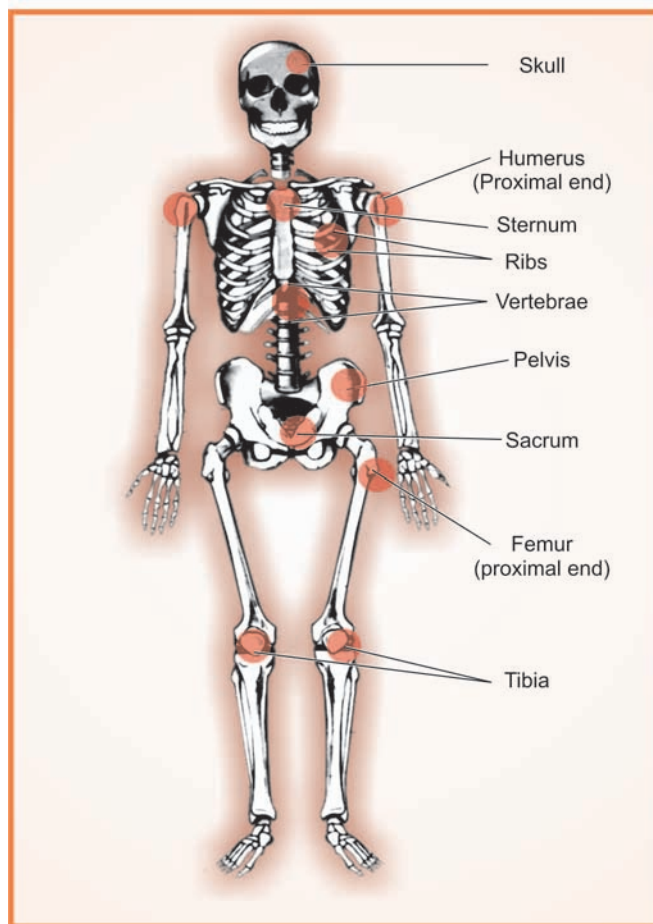


FIGURE 33.1

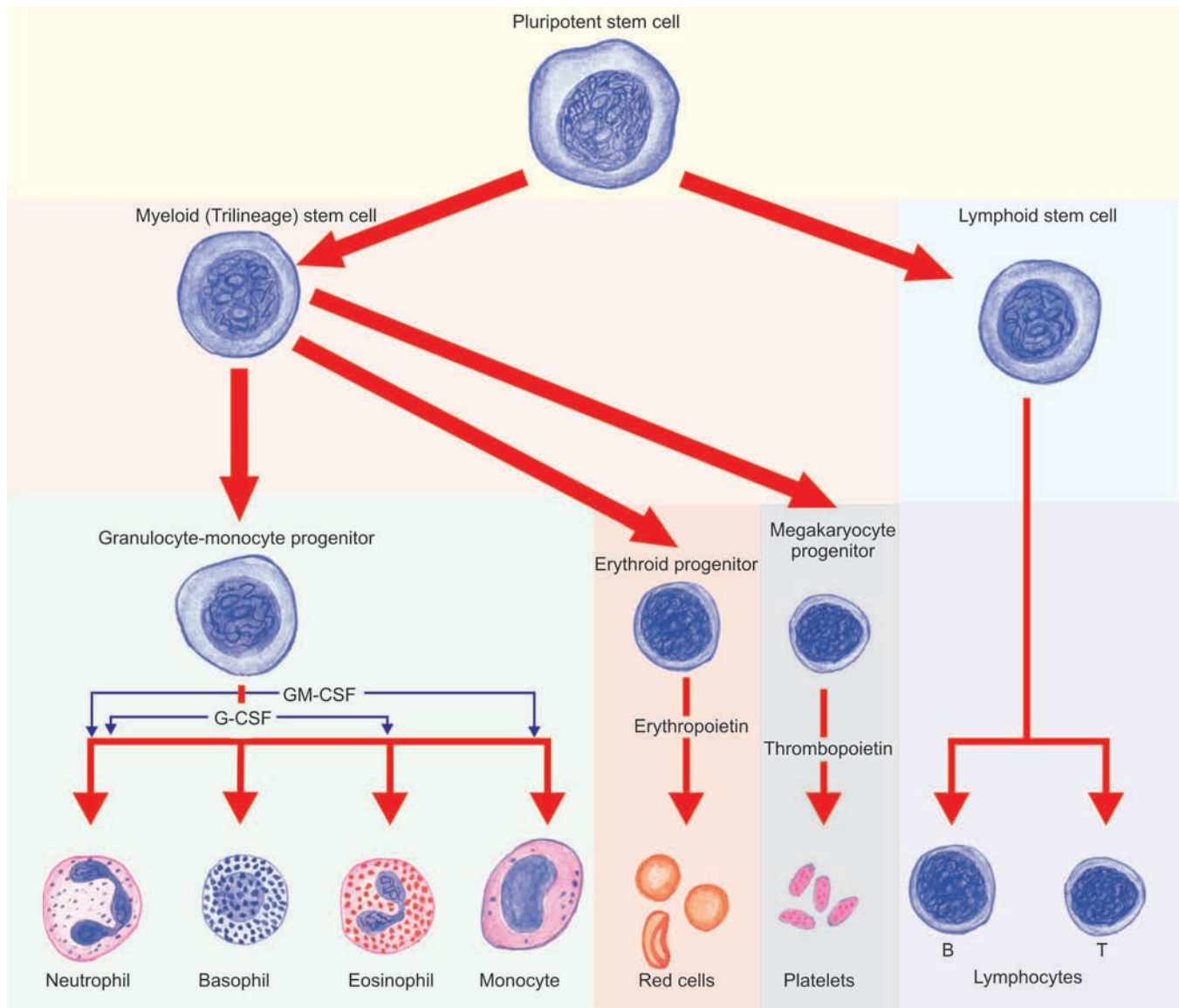
Sites of haematopoiesis in the bone marrow in the adult.

ment of mature cells (*-poiesis*)—red cells (erythropoiesis), granulocytes (granulopoiesis), monocytes, lymphocytes (lymphopoiesis) and platelets (thrombopoiesis) are considered in detail later in relevant sections.

Myeloid haematopoiesis or myelopoiesis includes differentiation and maturation of granulocytes, monocytes, erythroid cells and megakaryocytes (Fig. 33.2). The differentiation and maturation of these cells from stem cell are regulated by endogenous glycoproteins called as *growth factors, cytokines and hormones*. These are:

- Erythropoietin
- Granulocyte colony-stimulating factor (G-CSF)
- Granulocyte-macrophage colony-stimulating factor (GM-CSF)
- Thrombopoietin

Each of these growth factors act on specific receptors for growth factor to initiate further cell events as shown schematically in Fig. 33.2.

**FIGURE 33.2**

Schematic representation of differentiation of multipotent stem cells into blood cells.

BONE MARROW EXAMINATION

Examination of the bone marrow provides an invaluable diagnostic help in some cases, while in others it is of value in confirming a diagnosis suspected on clinical examination or on the blood film. A peripheral blood smear examination, however, must always precede bone marrow examination.

Bone marrow examination may be performed by two methods—*aspiration* and *trephine biopsy*. A comparison of the two methods is summarised in Table 33.1.

BONE MARROW ASPIRATION. The method involves suction of marrow via a strong, wide bore, short-bevelled needle fitted with a stylet and an adjustable guard in order to prevent excessive penetration; for instance Salah bone marrow aspiration needle. Smears are prepared immediately from the bone marrow aspirate and are fixed in 95% methanol after air-drying. The usual Romanowsky technique is employed for staining and a stain for iron is performed routinely so as to assess the reticuloendothelial stores of iron.

TABLE 33.1: Comparison of Bone Marrow Aspiration and Trephine Biopsy.

FEATURE	ASPIRATION	TREPINE
1. <i>Site</i>	Sternum, posterior iliac crest; tibial head in infants	Posterior iliac crest
2. <i>Instrument</i>	Salah BM aspiration needle	Jamshidi trephine needle
3. <i>Stains</i>	Romanowsky, Perls' reaction for iron on smears	Haematoxylin and eosin, reticulin on tissue sections
4. <i>Time</i>	Within 1-2 hours	Within 1-7 days
5. <i>Morphology</i>	Better cellular morphology of aspiration smears but marrow architecture is indistinct	Better marrow architectural pattern but cell morphology is not as distinct since tissue sections are examined and not smears
6. <i>Indications</i>	Anaemias, suspected leukaemias, neutropenia, thrombocytopenia, polycythaemia, myeloma, lymphomas, carcinomatosis, lipid storage diseases, granulomatous conditions, parasites, fungi, and unexplained enlargements of liver, spleen or lymph nodes.	Additional indications are: myelosclerosis, aplastic anaemia and in cases with 'dry tap' on aspiration.

The marrow film provides assessment of cellularity, details of developing blood cells (i.e. normoblastic or megaloblastic, myeloid, lymphoid, macrophages and megakaryocytic), ratio between erythroid and myeloid cells, storage diseases, and for the presence of cells foreign to the marrow such as secondary carcinoma, granulomatous conditions, fungi (e.g. histoplasmosis) and parasites (e.g. malaria, leishmaniasis, trypanosomiasis). Estimation of the proportion of cellular components in the marrow, however, can be provided by doing a differential count of at least 500 cells (myelogram, Table 33.2). In some conditions, the marrow cells can be used for more detailed special tests such as cytogenetics, microbiological culture, biochemical analysis, and immunological and cytological markers.

TREPINE BIOPSY. Trephine biopsy is performed by a simple *Jamshidi trephine needle* by which a core of tissue from periosteum to bone marrow cavity is obtained. The tissue is then fixed, decalcified and processed for histological sections and stained with haematoxylin and eosin and for reticulin. Trephine biopsy is useful over

aspiration since it provides an excellent view of the overall marrow architecture, cellularity, and presence or absence of infiltrates, but is less valuable than aspiration as far as individual cell morphology is concerned.

ERYTHROPOIESIS

Although the stem cells which eventually form the mature erythrocytes of the peripheral blood cannot be recognised morphologically, there is a well-defined and readily recognisable lineage of nucleated red cells, the erythroid series, in the marrow.

Erythroid Series

These cells are as under (Fig. 33.3):

1. **PRONORMOBLAST.** The earliest recognisable cell in the marrow is a pronormoblast or proerythroblast. It is a large cell, 15-20 µm in diameter having deeply basophilic cytoplasm and a large central nucleus containing nucleoli. The deep blue colour of the cytoplasm is due to high content of RNA which is associated with active protein synthesis. As the cells mature, the nuclei lose their nucleoli and become smaller and denser, while the cytoplasm on maturation leads to replacement of dense blue colour progressively by pink-staining haemoglobin. Each pronormoblast undergoes 4-5 replications and forms 16-32 mature RBCs.

2. **BASOPHILIC (EARLY) NORMOBLAST.** It is a round cell having a diameter of 12-16 µm with a large nucleus which is slightly more condensed than the pronormoblast and contains basophilic cytoplasm. Basophilic normoblast undergoes rapid proliferation.

TABLE 33.2: Normal Adult Bone Marrow Counts (Myelogram).

Fat/cell ratio : 50:50
Myeloid/erythroid (M/E) ratio : 2-4:1 (mean 3:1)
Myeloid series: 30-45% (37.5%)
• Myeloblasts : 0.1-3.5%
• Promyelocytes: 0.5-5%
Erythroid series: 10-15% (mean 12.5%)
Megakaryocytes: 0.5%
Lymphocytes: 5-20%
Plasma cells: ≤ 3%
Reticulum cells: 0.1-2%

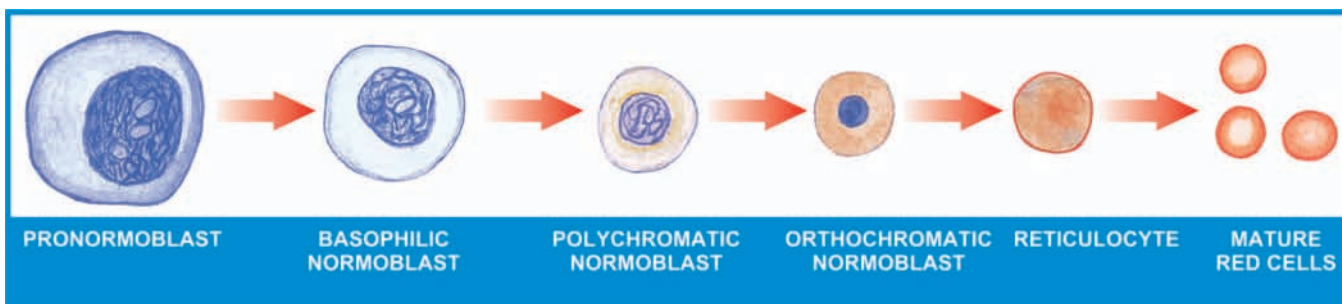


FIGURE 33.3

The erythroid series. There is progressive condensation of the nuclear chromatin which is eventually extruded from the cell at the late normoblast stage. The cytoplasm contains progressively less RNA and more haemoglobin.

3. POLYCHROMATIC (INTERMEDIATE) NORMOBLAST. Next maturation stage has a diameter of 12-14 μm . The nucleus at this stage is coarse and deeply basophilic. The cytoplasm is characteristically polychromatic i.e. contains admixture of basophilic RNA and acidophilic haemoglobin. The cell at this stage ceases to undergo proliferative activity.

4. ORTHOCHROMATIC (LATE) NORMOBLAST. The final stage in the maturation of nucleated red cells is the orthochromatic or late normoblast. The cell at this stage is smaller, 8-12 μm in diameter, containing a small and pyknotic nucleus with dark nuclear chromatin. The cytoplasm is characteristically acidophilic with diffuse basophilic hue due to the presence of large amounts of haemoglobin.

5. RETICULOCYTE. The nucleus is finally extruded from the late normoblast within the marrow and a reticulocyte results. The reticulocytes are juvenile red cells devoid of nucleus but contain ribosomal RNA so that they are still able to synthesise haemoglobin. A reticulocyte spends 1-2 days in the marrow and circulates for 1-2 days in the peripheral blood before maturing in the spleen, to become a biconcave red cell. The reticulocytes in the peripheral blood are distinguished from mature red cells by slightly basophilic hue in the cytoplasm similar to that of an orthochromatic normoblast. Reticulocytes can be counted in the laboratory by *vital staining* with dyes such as new methylene blue or brilliant cresyl blue. The reticulocytes by either of these staining methods contain deep blue reticulofilamentous material. While normoblasts are not normally present in human peripheral blood, reticulocytes are found normally in the peripheral blood. Normal range of reticulocyte count in health is 0.5-2.5% in adults and 2-6% in infants. Their percentage in the peripheral blood is a fairly accurate reflection of

erythropoietic activity. Their proportion is increased in conditions of rapid red cell regeneration e.g. after haemorrhage, haemolysis and haematopoietic response of anaemia to treatment.

Erythropoietin

Erythropoietic activity is regulated by the hormone, erythropoietin, which is produced in response to anoxia. The principal site of erythropoietin production is the kidney though there is evidence of its extra-renal production in certain unusual circumstances. Its levels are, therefore, lowered in chronic renal diseases, while a case of renal cell carcinoma may be associated with its enhanced production and erythrocytosis. Erythropoietin acts on the marrow at the various stages of morphologically unidentifiable as well as identifiable erythroid precursors.

There is an increased production of erythropoietin in various types of anaemias but in anaemia of chronic diseases (e.g. in infections and neoplastic conditions) there is no such enhancement of erythropoietin. In polycythaemia rubra vera, there is erythrocytosis but depressed production of erythropoietin. This is because of an abnormality of the stem cell class which is not under erythropoietin control.

The bioassay of erythropoietin in serum or urine is difficult due to quite low values. The alternative method for estimating erythropoietin concentration is *in vitro* method by induction of erythropoietin stimulation in mice.

The Red Cell

The mature erythrocytes of the human peripheral blood are non-nucleated cells and lack the usual cell organelles. The normal human erythrocyte is a biconcave disc, 7.2 μm in diameter, and has a thickness of 2.4 μm

at the periphery and 1 μm in the centre. The biconcave shape renders the red cells quite flexible so that they can pass through capillaries whose minimum diameter is 3.5 μm . More than 90% of the weight of erythrocyte consist of haemoglobin. The life-span of red cells is 120 days.

NORMAL VALUES AND RED CELL INDICES. Range of *normal red cell* count in health is $5.5 \pm 1.0 \times 10^{12}/\text{L}$ in men and $4.8 \pm 1.0 \times 10^{12}/\text{L}$ in women. The *packed cell volume* (PCV) or *haematocrit* is the volume of erythrocytes per litre of whole blood indicating the proportion of plasma and red cells and ranges $0.47 \pm 0.07 \text{ L/L}$ (40-54%) in men and $0.42 \pm 0.05 \text{ L/L}$ (37-47%) in women. The *haemoglobin content* in health is $15.5 \pm 2.5 \text{ g/dl}$ (13-18 g/dl) in men and $14.0 \pm 2.5 \text{ g/dl}$ (11.5-16.5 g/dl) in women. Based on these normal values, a series of *absolute values* or red cell indices can be derived which have diagnostic importance. These are as under:

1. Mean corpuscular volume (MCV) =

$$\frac{\text{PCV in L/L}}{\text{RBC count/L}}$$

The normal value is $85 \pm 8 \text{ fl}$ (77-93 fl)*.

2. Mean corpuscular haemoglobin (MCH) =

$$\frac{\text{Hb/L}}{\text{RBC count/L}}$$

The normal range is $29.5 \pm 2.5 \text{ pg}$ (27-32 pg)*.

3. Mean corpuscular haemoglobin concentration (MCHC) =

$$\frac{\text{Hb/dl}}{\text{PCV in L/L}}$$

The normal value is $32.5 \pm 2.5 \text{ g/dl}$ (30-35 g/dl).

Since MCHC is independent of red cell count and size, it is considered to be of greater clinical significance as compared to other absolute values. It is low in iron deficiency anaemia but is usually normal in macrocytic anaemia.

RED CELL MEMBRANE. The red cell membrane is a trilaminar structure having a bimolecular lipid layer interposed between two layers of proteins. The important *proteins* in red cell membrane are band 3 protein (named on the basis of the order in which it migrates during electrophoresis), glycophorin and spectrin;

important *lipids* are glycolipids, phospholipids and cholesterol; and *carbohydrates* form skeleton of erythrocytes having a lattice-like network which is attached to the internal surface of the membrane and is responsible for biconcave form of the erythrocytes.

A number of inherited disorders of the red cell membrane and cytoskeletal components produce abnormalities of the shape such as: *spherocytosis* (spherical shape from loss of part of the membrane), *ovalocytosis* (oval shape from loss of elasticity of cytoskeleton), *echinocytosis* (spiny processes from external surface due to metabolic abnormalities of red cells), and *stomatocytosis* (bowl-shaped red cells from expansion of inner membrane on one side).

NUTRITIONAL REQUIREMENTS FOR ERYTHROPOIESIS. New red cells are being produced each day for which the marrow requires certain essential substances. These substances are as under:

1. Metals. Iron is essential for red cell production because it forms part of the haem molecule in haemoglobin. Its deficiency leads to iron deficiency anaemia. Cobalt and manganese are certain other metals required for red cell production.

2. Vitamins. Vitamin B_{12} and folate are essential for biosynthesis of nucleic acids. Deficiency of B_{12} or folate causes megaloblastic anaemia. Vitamin C (ascorbic acid) plays an indirect role by facilitating the iron turnover in the body. Vitamin B_6 (pyridoxine), vitamin E (tocopherol) and riboflavin are the other essential vitamins required in the synthesis of red cells.

3. Amino acids. Amino acids comprise the globin component of haemoglobin. Severe amino acid deficiency due to protein deprivation causes depressed red cell production.

4. Hormones. As discussed above, erythropoietin plays a significant regulatory role in the erythropoietic activity. Besides erythropoietin, androgens and thyroxine also appear to be involved in the red cell production.

HAEMOGLOBIN. Haemoglobin consists of a basic protein, *globin*, and the iron-porphyrin complex, *haem*. The molecular weight of haemoglobin is 68,000. Normal adult haemoglobin (*HbA*) constitutes 96-98% of the total haemoglobin content and consists of four polypeptide chains, $\alpha_2 \beta_2$. Small quantities of 2 other haemoglobins present in adults are: *HbF* containing $\alpha_2 \gamma_2$ globin chains comprising 0.5-0.8% of total haemoglobin, and *HbA₂* having $\alpha_2 \delta_2$ chains and constituting 1.5-3.2% of total haemoglobin. Most of the haemoglobin (65%) is synthesised by the nucleated red cell precursors in the marrow, while the remainder (35%) is synthesised at the reticulocyte stage.

*For conversions, the multiples used are as follows: 'deci (d) = 10^{-1} , milli (m) = 10^{-3} , micro (μ) = 10^{-6} , nano (n) = 10^{-9} , pico (p) = 10^{-12} , femto (f) = 10^{-15}

Synthesis of haem occurs largely in the mitochondria by a series of biochemical reactions summarised in Fig. 33.4. Coenzyme, pyridoxal-6-phosphate, derived from pyridoxine (vitamin B₆) is essential for the synthesis of amino levulinic acid (ALA) which is the first step in the biosynthesis of protoporphyrin. The reaction is stimulated by erythropoietin and inhibited by haem. Ultimately, protoporphyrin combines with iron supplied from circulating transferrin to form haem. Each molecule of haem combines with a globin chain synthesised by polyribosomes. A tetramer of 4 globin chains, each having its own haem group, constitutes the haemoglobin molecule (Fig. 33.5, A).

RED CELL FUNCTIONS. The essential function of the red cells is to carry oxygen from the lungs to the tissue and to transport carbon dioxide to the lungs. In order to perform these functions, the red cells have the ability to generate energy as ATP by anaerobic glycolytic pathway (Embden-Meyerhof pathway). This pathway also generates reducing power as NADH and NADPH by the hexose monophosphate (HMP) shunt.

1. Oxygen carrying. The normal adult haemoglobin, HbA, is an extremely efficient oxygen-carrier. The four units of tetramer of haemoglobin molecule take up oxygen in succession, which, in turn, results in stepwise rise in affinity of haemoglobin for oxygen. This is responsible for the *sigmoid shape* of the oxygen dissociation curve.

The oxygen affinity of haemoglobin is expressed in term of P_{50} value which is the oxygen tension (pO_2) at which 50% of the haemoglobin is saturated with oxygen. Pulmonary capillaries have high pO_2 and, thus, there is virtual saturation of available oxygen-combining sites of haemoglobin. The tissue capillaries, however, have relatively low pO_2 and, thus, part of haemoglobin is in deoxy state. The extent to which oxygen is released from haemoglobin at pO_2 , in tissue capillaries depends upon 3 factors—the nature of globin chains, the pH, and the concentration of 2,3-biphosphoglycerate (2,3-BPG) as under (Fig 33.5, B).

■ Normal adult haemoglobin (HbA) has *lower affinity for oxygen* than foetal haemoglobin and, therefore, releases greater amount of bound oxygen at pO_2 of tissue capillaries.

■ A *fall in the pH* (acidic pH) lowers affinity of oxyhaemoglobin for oxygen, so called the Bohr effect, thereby causing enhanced release of oxygen from erythrocytes at the lower pH in tissue capillaries.

■ A *rise in red cell concentration of 2,3-BPG*, an intermediate product of Embden-Meyerhof pathway, as occurs in anaemia and hypoxia, causes decreased affinity of HbA for oxygen. This, in turn, results in enhanced supply of oxygen to the tissue.

2. CO₂ transport. Another important function of the red cells is the CO₂ transport. In the tissue capillaries, the pCO_2 is high so that CO₂ enters the erythrocytes

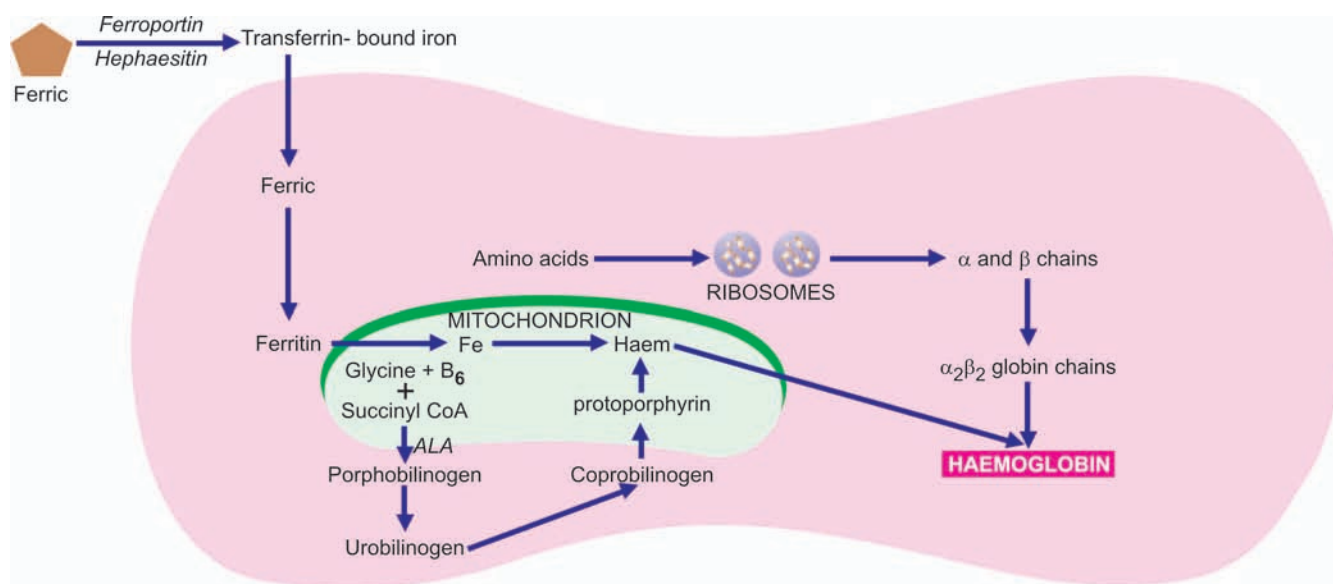


FIGURE 33.4

Schematic diagram of haemoglobin synthesis in the developing red cell.

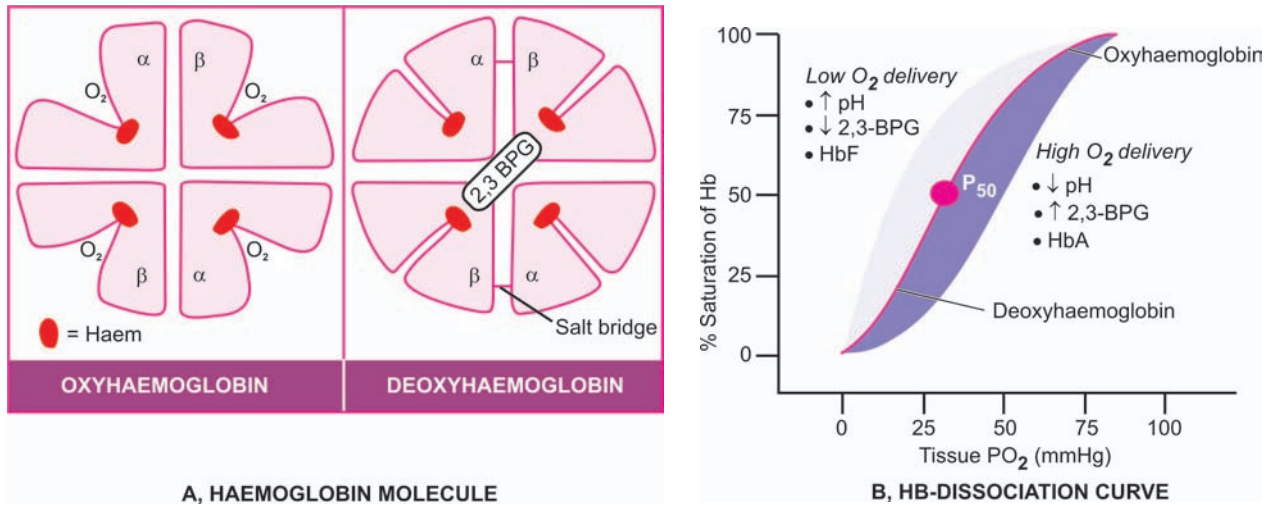


FIGURE 33.5

A, Normal adult haemoglobin molecule (HbA) consisting of $\alpha_2\beta_2$ globin chains, each with its own haem group in oxy and deoxy state. The haemoglobin tetramer can bind up to four molecules of oxygen in the iron containing sites of the haem molecules. As oxygen is bound, salt bridges are broken, and 2,3-BPG and CO_2 are expelled. B, Hb-dissociation curve. On dissociation of oxygen from Hb molecule i.e. on release of oxygen to the tissues, salt bridges are formed again, and 2,3-BPG and CO_2 are bound. The shift of the curve to higher oxygen delivery is affected by acidic pH, increased 2,3-BPG and HbA molecule while oxygen delivery is less with high pH, low 2,3-BPG and HbF.

where much of it is converted into bicarbonate ions which diffuse back into the plasma. In the pulmonary capillaries, the process is reversed and bicarbonate ions are converted back into CO_2 . Some of the CO_2 produced by tissues is bound to deoxyhaemoglobin forming carbamino-haemoglobin. This compound dissociates in the pulmonary capillaries to release CO_2 .

RED CELL DESTRUCTION. Red cells have a mean life-span of 120 days, after which red cell metabolism gradually deteriorates as the enzymes are not replaced. The destroyed red cells are removed mainly by the macrophages of the reticuloendothelial (RE) system of the marrow, and to some extent by the macrophages in the liver and spleen (Fig. 33.6). The breakdown of red cells liberates iron for recirculation via plasma transferrin to marrow normoblasts, and protoporphyrin which is broken down to bilirubin. Bilirubin circulates to the liver where it is conjugated to its diglucuronide which is excreted in the gut via bile and converted to stercobilinogen and stercobilin excreted in the faeces. Part of stercobilinogen and stercobilin is reabsorbed and excreted in the urine as urobilinogen and urobilin. A small fragment of protoporphyrin is converted to carbon monoxide and excreted in expired air from the lungs. Globin chains are broken down to amino acids and reused for protein synthesis in the body.

ANAEMIA—GENERAL CONSIDERATIONS

Anaemia is defined as a haemoglobin concentration in blood below the lower limit of the normal range for the age and sex of the individual. In adults, the lower extreme of the normal haemoglobin is taken as 13.0 g/dl for males and 11.5 g/dl for females. Newborn infants have higher haemoglobin level and, therefore, 15 g/dl is taken as the lower limit at birth, whereas at 3 months the lower level is 9.5 g/dl. Although haemoglobin value is employed as the major parameter for determining whether or not anaemia is present, the red cell counts, haematocrit (PCV) and absolute values (MCV, MCH and MCHC) provide alternate means of assessing anaemia.

Pathophysiology of Anaemia

Subnormal level of haemoglobin causes lowered oxygen-carrying capacity of the blood. This, in turn, initiates compensatory physiologic adaptations such as:

- increased release of oxygen from haemoglobin;
- increased blood flow to the tissues;
- maintenance of the blood volume; and
- redistribution of blood flow to maintain the cerebral blood supply.

Eventually, however, tissue hypoxia develops causing impaired functions of the affected tissues. The degree of functional impairment of individual tissues

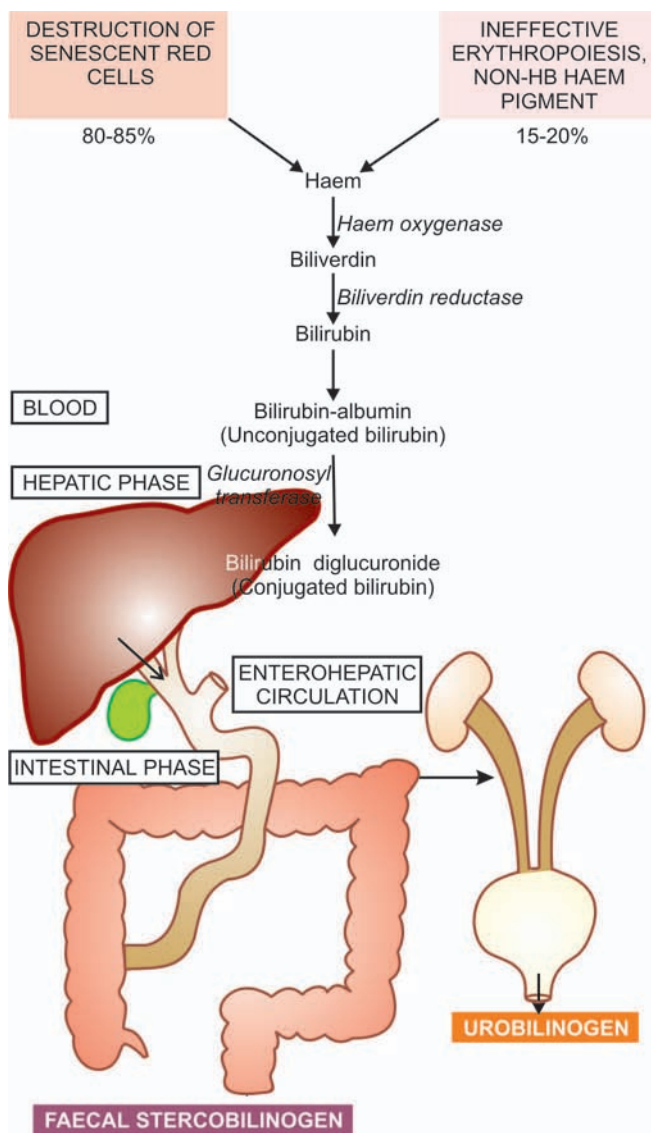


FIGURE 33.6

Normal red cell destruction in the RE system.

is variable depending upon their oxygen requirements. Tissues with high oxygen requirement such as the heart, CNS and the skeletal muscle during exercise, bear the brunt of clinical effects of anaemia.

Clinical Features of Anaemia

The haemoglobin level at which symptoms and signs of anaemia develop depends upon 4 main factors:

1. *The speed of onset of anaemia:* Rapidly progressive anaemia causes more symptoms than anaemia of slow onset as there is less time for physiologic adaptation.
2. *The severity of anaemia:* Mild anaemia produces no symptoms or signs but a rapidly developing severe

anaemia (haemoglobin below 6.0 g/dl) may produce significant clinical features.

3. *The age of the patient:* The young patients due to good cardiovascular compensation tolerate anaemia quite well as compared to the elderly. The elderly patients develop cardiac and cerebral symptoms more prominently due to associated cardiovascular disease.

4. *The haemoglobin dissociation curve:* In anaemia, the affinity of haemoglobin for oxygen is depressed as 2,3-BPG in the red cells increases. As a result, oxyhaemoglobin is dissociated more readily to release free oxygen for cellular use, causing a shift of the oxyhaemoglobin dissociation curve to the right.

SYMPTOMS. In symptomatic cases of anaemia, the presenting features are: tiredness, easy fatiguability, generalised muscular weakness, lethargy and headache. In older patients, there may be symptoms of cardiac failure, angina pectoris, intermittent claudication, confusion and visual disturbances.

SIGNS. A few general signs common to all types of anaemias are as under:

1. **Pallor.** Pallor is the most common and characteristic sign which may be seen in the mucous membranes, conjunctivae and skin.
2. **Cardiovascular system.** A hyperdynamic circulation may be present with tachycardia, collapsing pulse, cardiomegaly, midsystolic flow murmur, dyspnoea on exertion, and in the case of elderly, congestive heart failure.
3. **Central nervous system.** The older patients may develop symptoms referable to the CNS such as attacks of faintness, giddiness, headache, tinnitus, drowsiness, numbness and tingling sensations of the hands and feet.
4. **Ocular manifestations.** Retinal haemorrhages may occur if there is associated vascular disease or bleeding diathesis.
5. **Reproductive system.** Menstrual disturbances such as amenorrhoea and menorrhagia and loss of libido are some of the manifestations involving the reproductive system in anaemic subjects.
6. **Renal system.** Mild proteinuria and impaired concentrating capacity of the kidney may occur in severe anaemia.
7. **Gastrointestinal system.** Anorexia, flatulence, nausea, constipation and weight loss may occur.

In addition to the general features, specific signs may be associated with particular types of anaemia which are described later together with discussion of specific types of anaemias.

Investigations of the Anaemic Subject

After obtaining the full medical history pertaining to different general and specific signs and symptoms, the patient is examined for evidence of anaemia. Special emphasis is placed on colour of the skin, conjunctivae, sclerae and nails. Changes in the retina, atrophy of the papillae of the tongue, rectal examination for evidence of bleeding, and presence of hepatomegaly, splenomegaly, lymphadenopathy and bony tenderness are looked for.

In order to confirm or deny the presence of anaemia, its type and its cause, the following plan of investigations is generally followed.

A. HAEMOGLOBIN ESTIMATION. The first and foremost investigation in any suspected case of anaemia is to carry out a haemoglobin estimation. Several methods are available but most reliable and accurate is the cyanmethaemoglobin (HiCN) method employing Drabkin's solution and a spectrophotometer. If the haemoglobin value is below the lower limit of the normal range for particular age and sex, the patient is said to be anaemic. In pregnancy, there is haemodilution and, therefore, the lower limit in normal pregnant women is less (10.5 g/dl) than in the non-pregnant state.

B. PERIPHERAL BLOOD FILM EXAMINATION. The haemoglobin estimation is invariably followed by examination of a peripheral blood film for morphologic features after staining it with the Romanowsky dyes (e.g. Leishman's stain, May-Grünwald-Giemsa's stain, Jenner-Giemsa's stain etc). The blood smear is evaluated in an area where there is neither rouleaux formation nor so thin as to cause red cell distortion. Such an area can usually be found towards the tail of the film, but not actually at the tail. The following abnormalities in erythroid series of cells are particularly looked for in a blood smear:

1. Variation in size (Anisocytosis). Normally, there is slight variation in diameter of the red cells from 6.7-7.7 μm (mean value 7.2 μm). Increased variation in size of the red cell is termed anisocytosis. Anisocytosis may be due to the presence of cells larger than normal (*macrocytosis*) or cells smaller than normal (*microcytosis*). Sometimes both microcytosis and macrocytosis are present (*dimorphic*).

■ *Macrocytes* are classically found in megaloblastic anaemia; other causes are aplastic anaemia, other dyserythropoietic anaemias, chronic liver disease and in conditions with increased erythropoiesis.

■ *Microcytes* are present in iron deficiency anaemia, thalassaemia and spherocytosis. They may also result from fragmentation of erythrocytes such as in haemolytic anaemia.

2. Variation in shape (Poikilocytosis). Increased variation in shape of the red cells is termed poikilocytosis. The nature of the abnormal shape determines the cause of anaemia. Poikilocytes are produced in various types of abnormal erythropoiesis e.g. in megaloblastic anaemia, iron deficiency anaemia, thalassaemia, myelofibrosis and microangiopathic haemolytic anaemia.

3. Inadequate haemoglobin formation (Hypochromasia). Normally, the intensity of pink staining of haemoglobin in a Romanowsky-stained blood smear gradually decreases from the periphery to the centre of the cell. Increased central pallor is referred to as *hypochromasia*. It may develop either from lowered haemoglobin content (e.g. in iron deficiency anaemia, chronic infections), or due to thinness of the red cells (e.g. in thalassaemia, sideroblastic anaemia). Unusually deep pink staining of the red cells due to increased haemoglobin concentration is termed *hyperchromasia* and may be found in megaloblastic anaemia, spherocytosis and in neonatal blood.

4. Compensatory erythropoiesis. A number of changes are associated with compensatory increase in erythropoietic activity. These are as under:

i) *Polychromasia* is defined as the red cells having more than one type of colour. Polychromatic red cells are slightly larger, generally stained bluish-grey and represent reticulocytes and, thus, correlate well with reticulocyte count.

ii) *Normoblastaemia* is presence of nucleated red cells in the peripheral blood film. A small number of normoblasts (or erythroblasts) may be normally found in cord blood at birth. They are found in large numbers in haemolytic disease of the newborn, other haemolytic disorders and in extramedullary erythropoiesis. They may also appear in the blood in various types of severe anaemias except in aplastic anaemia. Normoblastaemia may also occur after splenectomy.

iii) *Punctate basophilia* or *basophilic stippling* is diffuse and uniform basophilic granularity in the cell which does not stain positively with Perls' reaction (in contrast to Pappenheimer bodies which stain positively). Classical punctate basophilia is seen in aplastic anaemia, thalassaemia, myelodysplasia, infections and lead poisoning.

iv) *Howell-Jolly bodies* are purple nuclear remnants, usually found singly, and are larger than basophilic stippling. They are present in megaloblastic anaemia and after splenectomy.

5. Miscellaneous changes. In addition to the morphologic abnormalities of red cells described above, several other abnormal red cells may be found in different haematological disorders. Some of these are as follows (Fig. 33.7):

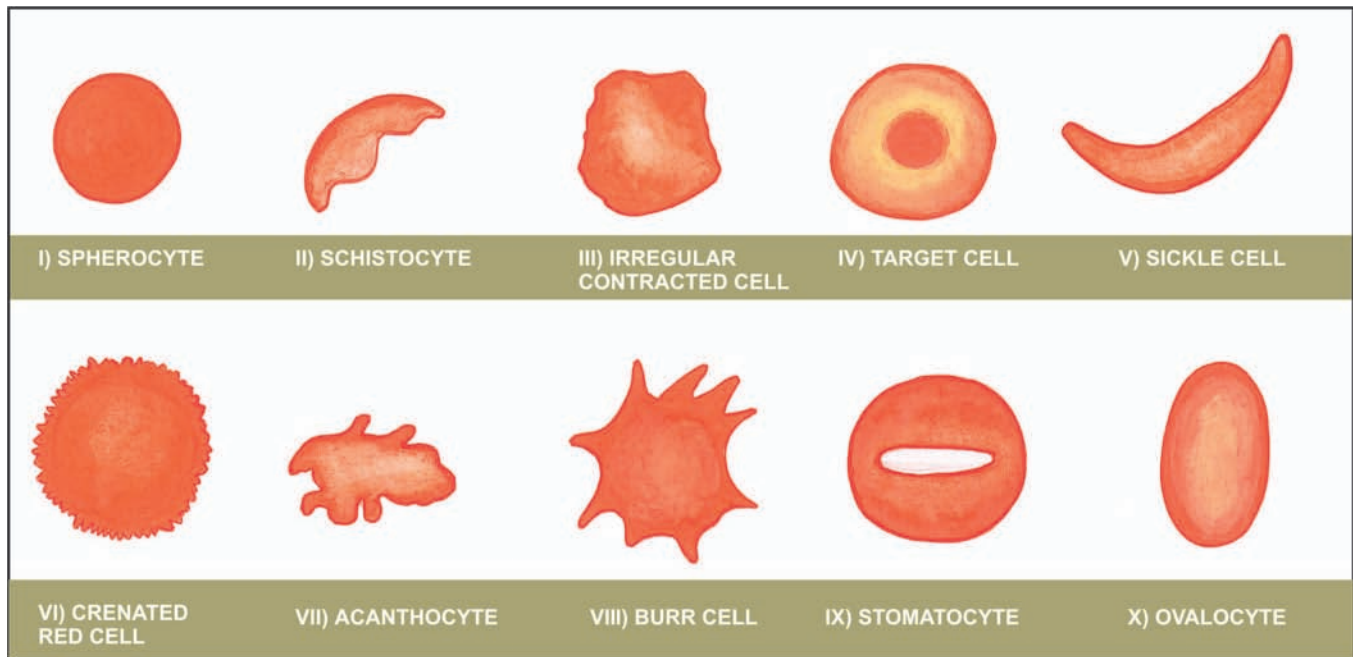


FIGURE 33.7

Some of the common morphologic abnormalities of red cells (The serial numbers in the illustrations correspond to the order in which they are described in the text).

i) *Spherocytosis* is characterised by presence of spheroidal rather than biconcave disc-shaped red cells. Spherocytes are seen in hereditary spherocytosis, autoimmune haemolytic anaemia and in ABO haemolytic disease of the newborn.

ii) *Schistocytosis* is identified by fragmentation of erythrocytes. Schistocytes are found in thalassaemia, hereditary elliptocytosis, megaloblastic anaemia, iron deficiency anaemia, microangiopathic haemolytic anaemia and in severe burns.

iii) *Irregularly contracted red cells* are found in drug and chemical induced haemolytic anaemia and in unstable haemoglobinopathies.

iv) *Leptocytosis* is the presence of unusually thin red cells. Leptocytes are seen in severe iron deficiency and thalassaemia. *Target cell* is a form of leptocyte in which there is central round stained area and a peripheral rim of haemoglobin. Target cells are found in iron deficiency, thalassaemia, chronic liver disease, and after splenectomy.

v) *Sickle cells or drepanocytes* are sickle-shaped red cells found in sickle cell disease.

vi) *Crenated red cells* are the erythrocytes which develop numerous projections from the surface. They are present

in blood films due to alkaline pH, presence of traces of fatty substances on the slides and in cases where the film is made from blood that has been allowed to stand overnight.

vii) *Acanthocytosis* is the presence of coarsely crenated red cells. Acanthocytes are found in large number in blood film made from splenectomised subjects, and in chronic liver disease.

viii) *Burr cells* are cell fragments having one or more spines. They are particularly found in uraemia.

ix) *Stomatocytosis* is the presence of stomatocytes which have central area having slit-like or mouth-like appearance. They are found in hereditary stomatocytosis, or may be seen in alcoholism.

x) *Ovalocytosis* or *elliptocytosis* is the oval or elliptical shape of red cells. Their highest proportion (79%) is seen in hereditary ovalocytosis and elliptocytosis; other conditions showing such abnormal shapes of red cells are megaloblastic anaemia and hypochromic anaemia.

C. RED CELL INDICES. An alternative method to diagnose and detect the severity of anaemia is by measuring the red cell indices.

■ In iron deficiency and thalassaemia, MCV, MCH and MCHC are reduced.

■ In anaemia due to acute blood loss and haemolytic anaemias, MCV, MCH and MCHC are all within normal limits.

■ In megaloblastic anaemias, MCV is raised above the normal range.

D. LEUCOCYTE AND PLATELET COUNT. Measurement of leucocyte and platelet count helps to distinguish pure anaemia from pancytopenia in which red cells, granulocytes and platelets are all reduced. In anaemias due to haemolysis or haemorrhage, the neutrophil count and platelet counts are often elevated. In infections and leukaemias, the leucocyte counts are high and immature leucocytes appear in the blood.

E. RETICULOCYTE COUNT. Reticulocyte count (normal 0.5-2.5%) is done in each case of anaemia to assess the marrow erythropoietic activity. In acute haemorrhage and in haemolysis, the reticulocyte response is indicative of impaired marrow function.

F. ERYTHROCYTE SEDIMENTATION RATE. The ESR is a non-specific test used as a screening test for anaemia. It usually gives a clue to the underlying organic disease but anaemia itself may also cause rise in the ESR.

G. BONE MARROW EXAMINATION. Bone marrow aspiration is done in cases where the cause for anaemia is not obvious. The procedures involved for marrow aspiration and trephine biopsy and their relative advantages and disadvantages have already been discussed (page 436).

In addition to these general tests, certain specific tests are done in different types of anaemias which are described later under the discussion of specific anaemias.

Classification of Anaemias

Several types of classifications of anaemias have been proposed. Two of the widely accepted classifications are based on the pathophysiology and morphology (Table 33.3).

PATHOPHYSIOLOGIC CLASSIFICATION. Depending upon the pathophysiological mechanism, anaemias are classified into 3 groups:

I. Anaemia due to blood loss. This is further of 2 types:

- A. Acute post-haemorrhagic anaemia
- B. Anaemia of chronic blood loss

II. Anaemia due to impaired red cell formation. A disturbance due to impaired red cell production from

TABLE 33.3: Classification of Anaemias.

A. PATHOPHYSIOLOGIC

I. Anaemia due to increased blood loss

- a) Acute post-haemorrhagic anaemia
- b) Chronic blood loss

II. Anaemias due to impaired red cell production

- a) *Cytoplasmic maturation defects*
 1. Deficient haem synthesis: Iron deficiency anaemia
 2. Deficient globin synthesis: Thalassaemic syndromes
- b) *Nuclear maturation defects*
Vitamin B₁₂ and/or folic acid deficiency: Megaloblastic anaemia
- c) *Defect in stem cell proliferation and differentiation*
 1. Aplastic anaemia
 2. Pure red cell aplasia
- d) *Anaemia of chronic disorders*
- e) *Bone marrow infiltration*
- f) *Congenital anaemia*

III. Anaemias due to increased red cell destruction (Haemolytic anaemias) (Details in Table 33.10)

- A. *Extrinsic (extracorporeal) red cell abnormalities*
- B. *Intrinsic (intracorporeal) red cell abnormalities*

B. MORPHOLOGIC

I. Microcytic, hypochromic

II. Normocytic, normochromic

III. Macrocytic, normochromic

various causes may produce anaemia. These are as under:

A. *Cytoplasmic maturation defects*

1. Deficient haem synthesis: iron deficiency anaemia
2. Deficient globin synthesis: thalassaemic syndromes

B. *Nuclear maturation defects*

Vitamin B₁₂ and/or folic acid deficiency: megaloblastic anaemia

C. *Haematopoietic stem cell proliferation and differentiation abnormality e.g.*

1. Aplastic anaemia
2. Pure red cell aplasia

D. *Bone marrow failure due to systemic diseases (anaemia of chronic disorders) e.g.*

1. Anaemia of inflammation/infections, disseminated malignancy
2. Anaemia in renal disease
3. Anaemia due to endocrine and nutritional deficiencies (hypometabolic states)
4. Anaemia in liver disease

E. *Bone marrow infiltration e.g.*

1. Leukaemias

2. Lymphomas
3. Myelosclerosis
4. Multiple myeloma

F. *Congenital anaemia* e.g.

1. Sideroblastic anaemia
2. Congenital dyserythropoietic anaemia

The term *hypoproliferative anaemias* is also used to denote impaired marrow proliferative activity and includes 2 main groups: hypoproliferation due to iron deficiency and that due to other hypoproliferative disorders; the latter category includes anaemia of chronic inflammation/infection, renal disease, hypometabolic states, and marrow damage.

III. Anaemia due to increased red cell destruction (haemolytic anaemias). This is further divided into 2 groups:

1. Intracorporeal defect (hereditary and acquired).
2. Extracorporeal defect (acquired haemolytic anaemias).

MORPHOLOGIC CLASSIFICATION. Based on the red cell size, haemoglobin content and red cell indices, anaemias are classified into 3 types:

1. **Microcytic, hypochromic:** MCV, MCH, MCHC are all reduced e.g. in iron deficiency anaemia and in certain non-iron deficient anaemias (sideroblastic anaemia, thalassaemia, anaemia of chronic disorders).
2. **Normocytic, normochromic:** MCV, MCH, MCHC are all normal e.g. after acute blood loss, haemolytic anaemias, bone marrow failure, anaemia of chronic disorders.
3. **Macrocytic:** MCV is raised e.g. in megaloblastic anaemia due to deficiency of vitamin B₁₂ or folic acid.

With these general comments on anaemias, a discussion of the specific types of anaemias is given in the following pages.

ANAEMIA OF BLOOD LOSS

Depending upon the rate of blood loss due to haemorrhage, the effects of post-haemorrhagic anaemia appear.

ACUTE BLOOD LOSS. When the loss of blood occurs suddenly, the following events take place:

- i) Immediate threat to life due to hypovolaemia which may result in shock and death.
- ii) If the patient survives, shifting of interstitial fluid to intravascular compartment with consequent haemodilution with low haematocrit.

iii) Hypoxia stimulates production of erythropoietin resulting in increased marrow erythropoiesis.

Laboratory Findings

- i) Normocytic and normochromic anaemia
- ii) Low haematocrit
- iii) Increased reticulocyte count in peripheral blood (10-15% after one week) reflecting accelerated marrow erythropoiesis.

CHRONIC BLOOD LOSS. When the loss of blood is slow and insidious, the effects of anaemia will become apparent only when the rate of loss is more than rate of production and the iron stores are depleted. This results in iron deficiency anaemia as seen in other clinical conditions discussed below.

HYPOCHROMIC ANAEMIAS

Iron deficiency is the commonest cause of anaemia the world over. It is estimated that about 20% of women in child-bearing age group are iron deficient, while the overall prevalence in adult males is about 2%. It is the most important, though not the sole, cause of microcytic hypochromic anaemia in which all the three red cell indices (MCV, MCH and MCHC) are reduced and occurs due to defective haemoglobin synthesis. Hypochromic anaemias, therefore, are classified into 2 groups:

- I. Hypochromic anaemia due to iron deficiency.
- II. Hypochromic anaemias other than iron deficiency.

The latter category includes 3 groups of disorders—sideroblastic anaemia, thalassaemia and anaemia of chronic disorders.

IRON DEFICIENCY ANAEMIA

The commonest nutritional deficiency disorder present throughout the world is iron deficiency but its prevalence is higher in the developing countries. The factors responsible for iron deficiency in different populations are variable and are best understood in the context of normal iron metabolism.

Iron Metabolism

The amount of iron obtained from the diet should replace the losses from the skin, bowel and genitourinary tract. These losses together are about 1 mg daily in an adult male or in a non-menstruating female, while in a menstruating woman there is an additional iron loss of 0.5-1 mg daily. The iron required for haemoglobin synthesis is derived from 2 primary sources—ingestion of

foods containing iron (e.g. leafy vegetables, beans, meats, liver etc) and recycling of iron from senescent red cells.

ABSORPTION. The average Western diet contains 10–15 mg of iron, out of which only 5–10% is normally absorbed. In pregnancy and in iron deficiency, the proportion of absorption is raised to 20–30%. Iron is absorbed mainly in the duodenum and proximal jejunum. *Iron from diet containing haem is better absorbed than non-haem iron.* Absorption of non-haem iron is enhanced by factors such as ascorbic acid (vitamin C), citric acid, amino acids, sugars, gastric secretions and hydrochloric acid. Iron absorption is impaired by factors like medicinal antacids, milk, pancreatic secretions, phytates, phosphates, ethylene diamine tetra-acetic acid (EDTA) and tannates contained in tea.

Non-haem iron is released as ferrous or ferric form but is absorbed almost exclusively as ferrous form; reduction when required takes place at the brush border by *ferrireductase*. Transport across the membrane is accomplished by divalent metal transporter 1 (DMT 1). Once inside the gut cells, ferric iron may be either stored as ferritin or further transported to transferrin by two vehicle proteins—*ferroportin* and *hephaestin*. The mechanism of dietary **haem iron** absorption is not quite clear yet. When the demand for iron is increased (e.g. during pregnancy, menstruation, periods of growth and various diseases), there is increased iron absorption, while excessive body stores of iron cause reduced intestinal iron absorption (see Fig. 33.11,A page 456).

DISTRIBUTION. In an adult, iron is distributed in the body as under:

1. **Haemoglobin**—present in the red cells, contains most of the body iron (65%).
2. **Myoglobin**—comprises a small amount of iron in the muscles (3.5%).
3. **Haem and non-haem enzymes**—e.g. cytochrome, catalase, peroxidases, succinic dehydrogenase and flavoproteins constitute a fraction of total body iron (0.5%).
4. **Transferrin-bound iron**—circulates in the plasma and constitutes another fraction of total body iron (0.5%).

All these forms of iron are in *functional form*.

5. **Ferritin and haemosiderin**—are the *storage forms* of excess iron (30%). They are stored in the mononuclear-phagocyte cells of the spleen, liver and bone marrow and in the parenchymal cells of the liver.

TRANSPORT. Iron is transported in plasma bound to a β -globulin, transferrin, synthesised in the liver. Transferrin-bound iron is made available to the marrow where

the immature red cell precursors utilise iron for haemoglobin synthesis. Transferrin is reutilised after iron is released from it. A small amount of transferrin iron is delivered to other sites such as parenchymal cells of the liver. Normally, transferrin is about one-third saturated. But in conditions where transferrin-iron saturation is increased, parenchymal iron uptake is increased. Virtually, no iron is deposited in the mononuclear-phagocyte cells (RE cells) from the plasma transferrin-iron but instead these cells derive most of their iron from phagocytosis of senescent red cells. Storage form of iron (ferritin and haemosiderin) in RE cells is normally not functional but can be readily mobilised in response to increased demands for erythropoiesis. However, conditions such as malignancy, infection and inflammation interfere with the release of iron from iron stores causing ineffective erythropoiesis.

EXCRETION. The body is unable to regulate its iron content by excretion alone. The amount of iron lost per day is 0.5–1 mg which is independent of iron intake. This loss is nearly twice more (i.e. 1–2 mg/day) in menstruating women. Iron is lost from the body as a result of desquamation of epithelial cells from the gastrointestinal tract, from excretion in the urine and sweat, and loss via hair and nails. Iron excreted in the faeces mainly consists of unabsorbed iron and desquamated mucosal cells.

The daily iron cycle in the body is diagrammatically summarised in Fig. 33.8.

Pathogenesis

Iron deficiency anaemia develops when the supply of iron is inadequate for the requirement of haemoglobin synthesis. Initially, the negative iron balance is made good by mobilisation from the tissue stores so as to maintain haemoglobin synthesis. It is only after the tissue stores of iron are exhausted that the supply of iron to the marrow becomes insufficient for haemoglobin formation so that a state of iron deficiency anaemia develops. The development of iron deficiency depends upon one or more of the following factors:

1. Increased blood loss
2. Increased requirements
3. Inadequate dietary intake
4. Decreased intestinal absorption.

The relative significance of these factors varies with the age and sex of the patient (Table 33.4). Accordingly, certain groups of individuals at increased risk of developing iron deficiency can be identified (*see below*). In general, in developed countries the mechanism of iron deficiency is usually due to chronic occult blood

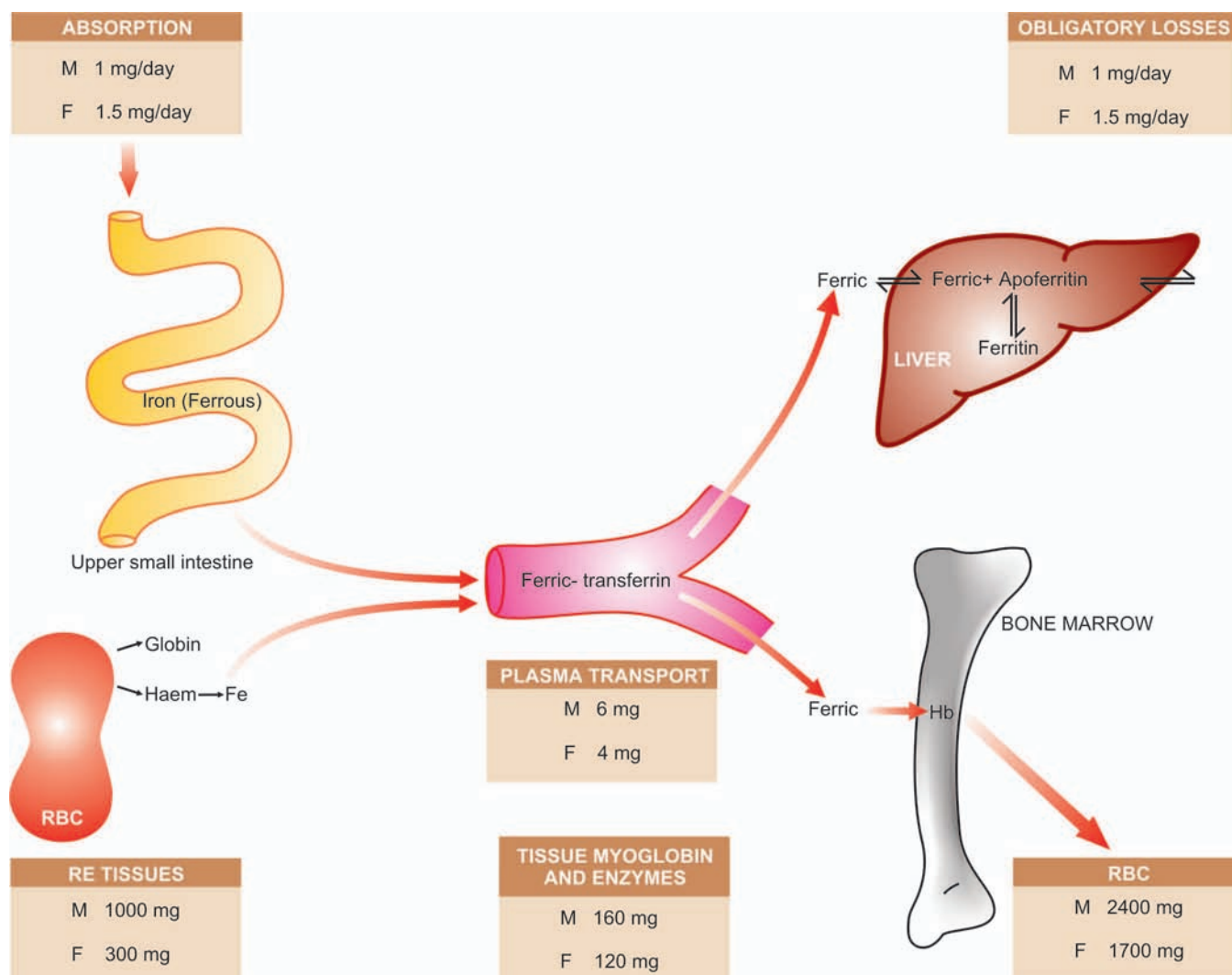


FIGURE 33.8

Daily iron cycle. Iron on absorption from upper small intestine circulates in plasma bound to transferrin and is transported to the bone marrow for utilisation in haemoglobin synthesis. The mature red cells are released into circulation, which on completion of their life-span of 120 days, die. They are then phagocytosed by RE cells and iron stored as ferritin and haemosiderin. Stored iron is mobilised in response to increased demand and used for haemoglobin synthesis, thus completing the cycle (M = males; F = females).

loss, while in the underdeveloped countries poor intake of iron or defective absorption are responsible for iron deficiency anaemia.

Etiology

Iron deficiency anaemia is always secondary to an underlying disorder. Correction of the underlying cause, therefore, is essential part of its treatment. Based on the above-mentioned pathogenetic mechanisms, the following etiologic factors are involved in development

of iron deficiency anaemia at different age and sex (Table 33.4):

1. FEMALES IN REPRODUCTIVE PERIOD OF LIFE.

The highest incidence of iron deficiency anaemia is in women during their reproductive period of life. It may be from one or more of the following causes:

i) *Blood loss.* This is the most important cause of anaemia in women during child-bearing age group. Commonly, it is due to persistent and heavy menstrual blood loss such as occurs in various pathological states

TABLE 33.4: Etiology of Iron Deficiency Anaemia.

I. INCREASED BLOOD LOSS

1. *Uterine* e.g. excessive menstruation in reproductive years, repeated miscarriages, at onset of menarche, post-menopausal uterine bleeding.
2. *Gastrointestinal* e.g. peptic ulcer, haemorrhoids, hookworm infestation, cancer of stomach and large bowel, oesophageal varices, hiatus hernia, chronic aspirin ingestion, ulcerative colitis, diverticulosis.
3. *Renal tract* e.g. haematuria, haemoglobinuria.
4. *Nose* e.g. repeated epistaxis.
5. *Lungs* e.g. haemoptysis.

II. INCREASED REQUIREMENTS

1. Spurts of growth in infancy, childhood and adolescence.
2. Prematurity.
3. Pregnancy and lactation.

III. INADEQUATE DIETARY INTAKE

1. Poor economic status.
2. Anorexia e.g. in pregnancy.
3. Elderly individuals due to poor dentition, apathy and financial constraints.

IV. DECREASED ABSORPTION

1. Partial or total gastrectomy
2. Achlorhydria
3. Intestinal malabsorption such as in coeliac disease.

and due to insertion of IUCDs. Young girls at the onset of menstruation may develop mild anaemia due to blood loss. Significant blood loss may occur as a result of repeated miscarriages.

ii) *Inadequate intake*. Inadequate intake of iron is prevalent in women of lower economic status. Besides diet deficient in iron, other factors such as anorexia, impaired absorption and diminished bioavailability may act as contributory factors.

iii) *Increased requirements*. During pregnancy and adolescence, the demand of body for iron is increased. During a normal pregnancy, about 750 mg of iron may be siphoned off from the mother—about 400 mg to the foetus, 150 mg to the placenta, and 200 mg is lost at parturition and lactation. If several pregnancies occur at short intervals, iron deficiency anaemia certainly follows.

2. POST-MENOPAUSAL FEMALES. Though the physiological demand for iron decreases after cessation of menstruation, iron deficiency anaemia may develop in post-menopausal women due to chronic blood loss. Among the important causes are:

i) *Post-menopausal uterine bleeding* due to carcinoma of the uterus.

ii) *Bleeding from the alimentary tract* such as due to carcinoma of stomach and large bowel and hiatus hernia.

3. ADULT MALES. It is uncommon for adult males to develop iron deficiency anaemia in the presence of normal dietary iron content and iron absorption. The vast majority of cases of iron deficiency anaemia in adult males are due to chronic blood loss. The cause for chronic haemorrhage may lie at one of the following sites:

i) *Gastrointestinal tract* is the usual source of bleeding which may be due to peptic ulcer, haemorrhoids, hookworm infestation, carcinoma of stomach and large bowel, oesophageal varices, hiatus hernia, chronic aspirin ingestion and ulcerative colitis. Other causes in the GIT are malabsorption and following gastrointestinal surgery.

ii) *Urinary tract* e.g. due to haematuria and haemoglobinuria.

iii) *Nose* e.g. in repeated epistaxis.

iv) *Lungs* e.g. in haemoptysis from various causes.

4. INFANTS AND CHILDREN. Iron deficiency anaemia is fairly common during infancy and childhood with a peak incidence at 1-2 years of age. The principal cause for anaemia at this age is increased demand of iron which is not met by the inadequate intake of iron in the diet. The normal full-term infant has sufficient iron stores for the first 4-6 months of life, while premature infants have inadequate reserves because iron stores from the mother are mainly laid down during the last trimester of pregnancy. Therefore, unless the infant is given supplemental feeding of iron or iron-containing foods, iron deficiency anaemia develops.

Clinical Features

As already mentioned, iron deficiency anaemia is much more common in women between the age of 20 and 45 years than in men; at periods of active growth in infancy, childhood and adolescence; and is also more frequent in premature infants. Initially, there are usually no clinical abnormalities. But subsequently, in addition to features of the underlying disorder causing the anaemia, the clinical consequences of iron deficiency manifest in 2 ways—anaemia itself and epithelial tissue changes.

1. ANAEMIA. The onset of iron deficiency anaemia is generally slow. The usual symptoms are of weakness, fatigue, dyspnoea on exertion, palpitations and pallor of the skin, mucous membranes and sclerae. Older patients may develop angina and congestive cardiac failure. Patients may have unusual dietary cravings such

as pica. Menorrhagia is a common symptom in iron deficient women.

2. EPITHELIAL TISSUE CHANGES. Long-standing chronic iron deficiency anaemia causes epithelial tissue changes in some patients. The changes occur in the nails (koilonychia or spoon-shaped nails), tongue (atrophic glossitis), mouth (angular stomatitis), and oesophagus causing dysphagia from development of thin, membranous webs at the postcricoid area (Plummer-Vinson syndrome).

Laboratory Findings

The development of anaemia progresses in 3 stages:

■ Firstly, *storage iron depletion* occurs during which iron reserves are lost without compromise of the iron supply for erythropoiesis.

■ The next stage is *iron deficient erythropoiesis* during which the erythroid iron supply is reduced without the development of anaemia.

■ The final stage is the development of *frank iron deficiency anaemia* when the red cells become microcytic and hypochromic.

The following laboratory tests can be used to assess the varying degree of iron deficiency (Fig. 33.9):

1. BLOOD PICTURE AND RED CELL INDICES.

The degree of anaemia varies. It is usually mild to moderate but occasionally it may be marked

(haemoglobin less than 6 g/dl) due to persistent and severe blood loss. The salient haematological findings in these cases are as under (**COLOUR PLATE X: CL 37**):

i) Haemoglobin. The essential feature is a fall in haemoglobin concentration up to a variable degree.

ii) Red cells. The red cells in the blood film are hypochromic and microcytic, and there is anisocytosis and poikilocytosis. Hypochromia generally precedes microcytosis. Hypochromia is due to poor filling of the red cells with haemoglobin so that there is increased central pallor. In severe cases, there may be only a thin rim of pink staining at the periphery. Target cells, elliptical forms and polychromatic cells are often present. Normoblasts are uncommon. RBC count is below normal but is generally not proportionate to the fall in haemoglobin value. When iron deficiency is associated with severe folate or vitamin B₁₂ deficiency, a *dimorphic* blood picture occurs with dual population of red cells—macrocytic as well as microcytic hypochromic.

iii) Reticulocyte count. The reticulocyte count is normal or reduced but may be slightly raised (2-5%) in cases after haemorrhage.

iv) Absolute values. The red cell indices reveal a diminished MCV (below 50 fl), diminished MCH (below 15 pg), and diminished MCHC (below 20 g/dl).

v) Leucocytes. The total and differential white cell counts are usually normal.

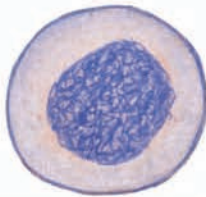
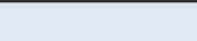
LABORATORY FINDINGS		NORMAL	IRON-DEFICIENCY ANAEMIA
BLOOD	RED CELL MORPHOLOGY	 Normal red cell	 Microcytic hypochromic red cell
	RED CELL INDICES	MCV, MCH, MCHC all normal	MCV ↓, MCH ↓, MCHC ↓
BONE MARROW	MARROW ERYTHROPOIESIS	 Normoblastic	 Micronormoblastic
	MARROW IRON STORES	 Normal	 Deficient

FIGURE 33.9

Laboratory findings in iron deficiency anaemia.

vi) Platelets. Platelet count is usually normal but may be slightly to moderately raised in patients who have had recent bleeding.

2. BONE MARROW FINDINGS. Bone marrow examination is not essential in such cases routinely but is done in complicated cases so as to distinguish from other hypochromic anaemias. The usual findings are as follows:

i) Marrow cellularity. The marrow cellularity is increased due to erythroid hyperplasia (myeloid-erythroid ratio decreased).

ii) Erythropoiesis. There is normoblastic erythropoiesis with predominance of small polychromatic normoblasts (micronormoblasts). These normoblasts have a thin rim of cytoplasm around the nucleus and a ragged and irregular cell border. The *cytoplasmic maturation lags behind* so that the late normoblasts have pyknotic nucleus but persisting polychromatic cytoplasm (compared from megaloblastic anaemia in which the nuclear maturation lags behind, page 460).

iii) Other cells. Myeloid, lymphoid and megakaryocytic cells are normal in number and morphology.

iv) Marrow iron. Iron staining (Prussian blue reaction) carried out on bone marrow aspirate smear shows deficient reticuloendothelial iron stores and absence of siderotic iron granules from developing normoblasts.

3. BIOCHEMICAL FINDINGS. In addition to blood and bone marrow examination, the following biochemical tests are of value:

i) The *serum iron* level is low (normal 80-180 µg/dl); it is often under 50 µg/dl.

ii) *Total iron binding capacity (TIBC)* is high (normal 250-450 µg/dl) and rises to give less than 10% saturation (normal 33%). In anaemia of chronic disorders, serum iron as well as TIBC are reduced.

iii) The *serum ferritin* is very low (normal 150-2000 ng/dl) indicating poor tissue iron stores. The serum ferritin is raised in iron overload and is normal in anaemia of chronic disorders.

iv) The *red cell protoporphyrin* is very low (normal 20-40 µg/dl) as a result of insufficient iron supply to form haem.

1. CORRECTION OF THE DISORDER. The underlying cause of iron deficiency is established after thorough check-up and investigations. Appropriate surgical, medical or preventive measures are instituted to correct the cause of blood loss.

2. CORRECTION OF IRON DEFICIENCY. The lack of iron is corrected with iron therapy as under:

i) Oral therapy. Iron deficiency responds very effectively to the administration of oral iron salts such as ferrous sulfate. One tablet containing 60 mg of elemental iron is administered thrice daily. Optimal absorption is obtained by giving iron fasting, but if side-effects occur (e.g. nausea, abdominal discomfort, diarrhoea) iron can be given with food or by using a preparation of lower iron content (e.g. ferrous gluconate containing 37 mg elemental iron). Oral iron therapy is continued long enough, both to correct the anaemia and to replenish the body iron stores. The response to oral iron therapy is observed by reticulocytosis which begins to appear in 3-4 days with a peak in about 10 days. Poor response to iron replacement may occur from various causes such as: incorrect diagnosis, non-compliance, continuing blood loss, bone marrow suppression by tumour or chronic inflammation, and malabsorption.

ii) Parenteral therapy. Parenteral iron therapy is indicated in cases who are intolerant to oral iron therapy, in GIT disorders such as malabsorption, or a rapid replenishment of iron stores is desired such as in women with severe anaemia a few weeks before expected date of delivery. Parenteral iron therapy is hazardous and expensive when compared with oral administration. The haematological response to parenteral iron therapy is no faster than the administration of adequate dose of oral iron but the stores are replenished much faster. Before giving the parenteral iron, total dose is calculated by a simple formula by multiplying the grams of haemoglobin below normal with 250 (250 mg of elemental iron is required for each gram of deficit haemoglobin). It may be given as a single intramuscular injection of iron dextran (imferon), or repeated injections of iron-sorbitol citrate (jectofer). The adverse effects include hypersensitivity or anaphylactoid reactions, haemolysis, hypotension, circulatory collapse, vomiting and muscle pain. A recently introduced preparation, iron gluconate, is given intravenously.

SIDEROBLASTIC ANAEMIA

The sideroblastic anaemias comprise a group of disorders of diverse etiology in which the nucleated erythroid precursors in the bone marrow, show characteristic 'ringed sideroblasts.'

Treatment

The management of iron deficiency anaemia consists of 2 essential principles: correction of disorder causing the anaemia, and correction of iron deficiency.

Siderocytes and Sideroblasts

Siderocytes and sideroblasts are erythrocytes and normoblasts respectively which contain cytoplasmic granules of iron (Fig. 33.10).

SIDEROCYTES. These are red cells containing granules of non-haem iron. These granules stain positively with Prussian blue reaction as well as stain with Romanowsky dyes when they are referred to as *Pappenheimer bodies*. Siderocytes are normally not present in the human peripheral blood but a small number may appear following splenectomy. This is because the reticulocytes on release from the marrow are finally sequestered in the spleen to become mature red cells. In the absence of spleen, the final maturation step takes place in the peripheral blood and hence the siderocytes make their appearance in the blood after splenectomy.

SIDEROBLASTS. These are nucleated red cells (normoblasts) containing siderotic granules which stain positively with Prussian blue reaction. Depending upon the number, size and distribution of siderotic granules, sideroblasts may be normal or abnormal (Fig. 33.10).

Normal sideroblasts contain a few fine, scattered cytoplasmic granules representing iron which has not been utilised for haemoglobin synthesis. These cells comprise 30-50% of normoblasts in the normal marrow but are reduced or absent in iron deficiency.

Abnormal sideroblasts are further of 2 types:

■ *One type* is a sideroblast containing numerous, diffusely scattered, coarse cytoplasmic granules and are seen in conditions such as dyserythropoiesis and haemolysis. In this type, there is no defect of haem or globin synthesis but the percentage saturation of transferrin is increased.

■ The other type is *ringed sideroblast* in which haem synthesis is disturbed as occurs in sideroblastic anaemias. Ringed sideroblasts contain numerous large granules, often forming a complete or partial ring around the nucleus. The ringed arrangement of these granules is due to the presence of iron-laden mitochondria around the nucleus.

Types of Sideroblastic Anaemias

Based on etiology, sideroblastic anaemias are classified into hereditary and acquired types. The acquired type is further divided into primary and secondary forms (Table 33.5).

I. HEREDITARY SIDEROBLASTIC ANAEMIA. This is a rare X-linked disorder associated with defective enzyme activity of *aminolevulinic acid (ALA) synthetase* required for haem synthesis. The affected males have

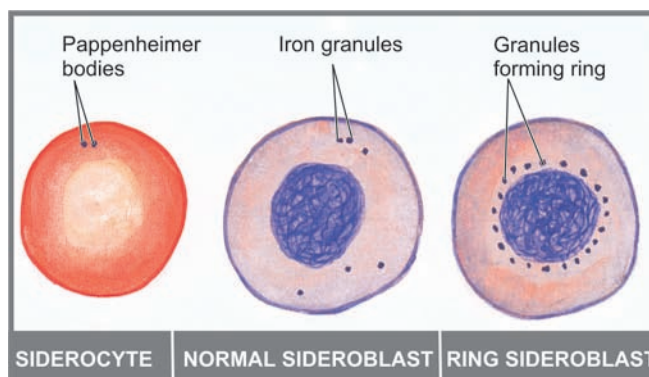


FIGURE 33.10

A siderocyte containing Pappenheimer bodies, a normal sideroblast and a ring sideroblast.

moderate to marked anaemia while the females are carriers of the disorder and do not develop anaemia. The condition manifests in childhood or in early adult life.

II. ACQUIRED SIDEROBLASTIC ANAEMIA. The acquired sideroblastic anaemias are classified into primary and secondary types.

A. Primary acquired sideroblastic anaemia. Primary, idiopathic, or refractory acquired sideroblastic anaemia occurs spontaneously in middle-aged and older individuals of both sexes. The disorder has its pathogenesis in disturbed growth and maturation of erythroid precursors at the level of haematopoietic stem cell, possibly due to reduced activity of the enzyme, ALA synthetase. The anaemia is of moderate to severe degree and appears insidiously. The bone marrow cells commonly show chromosomal abnormalities, neutropenia and thrombocytopenia with associated bleeding diathesis. The spleen and liver may be either normal or mildly enlarged, while the lymph nodes are not enlarged. Unlike other types of sideroblastic anaemia,

TABLE 33.5: Classification of Sideroblastic Anaemia.

I. HEREDITARY (CONGENITAL) SIDEROBLASTIC ANAEMIA
II. ACQUIRED SIDEROBLASTIC ANAEMIA
A. Primary (idiopathic, refractory) acquired sideroblastic anaemia
B. Secondary acquired sideroblastic anaemia
1. <i>Drugs, chemicals and toxins</i> e.g. isoniazid, cycloserine, chloramphenicol, cyclophosphamide, alcohol and lead.
2. <i>Haematological disorders</i> e.g. myelofibrosis, polycythaemia vera, acute leukaemia, myeloma, lymphoma and haemolytic anaemia.
3. <i>Miscellaneous</i> e.g. carcinoma, myxoedema, rheumatoid arthritis and SLE.

this type is regarded as a myelodysplastic disorder in the FAB (French-American-British) classification and thus, can be a preleukaemic disorder (page 480). About 10% of individuals with refractory acquired sideroblastic anaemia develop acute myelogenous leukaemia.

B. Secondary acquired sideroblastic anaemia. Acquired sideroblastic anaemia may develop secondary to a variety of drugs, chemicals, toxins, haematological and various other diseases.

1. *Drugs, chemicals and toxins:* Isoniazid, an anti-tuberculous drug and a pyridoxine antagonist, is most commonly associated with development of sideroblastic anaemia by producing abnormalities in pyridoxine metabolism. Other drugs occasionally causing acquired sideroblastic anaemia are: cycloserine, chloramphenicol and alkylating agents (e.g. cyclophosphamide). Alcohol and lead also cause sideroblastic anaemia. All these agents cause reversible sideroblastic anaemia which usually resolves following removal of the offending agent.

2. *Haematological disorders:* These include myelofibrosis, polycythaemia vera, acute leukaemia, myeloma, lymphoma and haemolytic anaemia.

3. *Miscellaneous:* Occasionally, secondary sideroblastic anaemia may occur in association with a variety of inflammatory, neoplastic and autoimmune diseases such as carcinoma, myxoedema, rheumatoid arthritis and SLE.

Laboratory Findings

Sideroblastic anaemias usually show the following haematological features:

1. There is generally moderate to severe degree of *anaemia*.
2. The *blood picture* shows hypochromic anaemia which may be microcytic, or there may be some normocytic red cells as well (dimorphic).

3. *Absolute values* (MCV, MCH and MCHC) are reduced in hereditary type but MCV is often raised in acquired type.

4. The *bone marrow examination* shows erythroid hyperplasia with usually macronormoblastic erythropoiesis. Marrow iron stores are raised and pathognomonic ring sideroblasts are present.

5. *Serum ferritin* levels are raised.

6. *Serum iron* is usually raised with almost complete saturation of TIBC.

7. There is increased *iron deposition* in the tissue.

Treatment

The treatment of secondary sideroblastic anaemia is primarily focussed on removal of the offending agent. No definite treatment is available for hereditary and idiopathic types of sideroblastic anaemias. However, pyridoxine is administered routinely to all cases of sideroblastic anaemia (200 mg per day for 2-3 months). Blood transfusions and other supportive therapy are indicated in all patients. More recently, clinical trials have been attempted by giving cytokines such as erythropoietin, colony-stimulating factor and interleukin-3.

Differential diagnosis of various types of hypochromic anaemias by laboratory tests is summarised in Table 33.6.

ANAEMIA OF CHRONIC DISORDERS

One of the commonly encountered anaemia is in patients of a variety of chronic systemic diseases in which anaemia develops secondary to disease process but there is no actual invasion of the bone marrow. A list of such chronic systemic diseases is given in Table 33.7. In general, the anaemia in chronic disorders is usually normocytic normochromic but can have mild degree of

TABLE 33.6: Laboratory Diagnosis of Hypochromic Anaemias.

TEST	IRON DEFICIENCY	CHRONIC DISORDERS	THALASSAEMIA	SIDEROBLASTIC ANAEMIA
1. MCV, MCH, MCHC	Reduced	Low normal-to-reduced	Very low	Very low (except MCV raised in acquired type)
2. Serum iron	Reduced	Reduced	Normal	Raised
3. TIBC	Raised	Reduced	Normal	Normal
4. Serum ferritin	Reduced	Raised	Normal	Raised (complete saturation)
5. Marrow-iron stores	Absent	Present	Present	Present
6. Iron in normoblasts	Absent	Absent	Present	Ring sideroblasts
7. Hb electrophoresis	Normal	Normal	Abnormal	Normal

TABLE 33.7: Anaemias Secondary to Chronic Systemic Disorders.

1. **ANAEMIA IN CHRONIC INFECTIONS/INFLAMMATION**
 - a. *Infections* e.g. tuberculosis, lung abscess, pneumonia, osteomyelitis, subacute bacterial endocarditis, pyelonephritis.
 - b. *Non-infectious inflammations* e.g. rheumatoid arthritis, SLE, vasculitis, dermatomyositis, scleroderma, sarcoidosis, Crohn's disease.
 - c. *Disseminated malignancies* e.g. Hodgkin's disease, disseminated carcinomas and sarcomas.
2. **ANAEMIA OF RENAL DISEASE** e.g. uraemia, renal failure
3. **ANAEMIA OF HYPOMETABOLIC STATE** e.g. endocrinopathies (myxoedema, Addison's disease, hyperthyroidism, hypopituitarism, Addison's disease), protein malnutrition, scurvy and pregnancy, liver disease.

microcytosis and hypochromia unrelated to iron deficiency. The severity of anaemia is usually directly related to the primary disease process. The anaemia is corrected only if the primary disease is alleviated.

Pathogenesis

A number of factors may contribute to the development of anaemia in chronic systemic disorders, and in many conditions, the anaemia is complicated by other causes such as iron, B₁₂ and folate deficiency, hypersplenism, renal failure with consequent reduced erythropoietic activity, endocrine abnormalities etc. However, in general, 2 factors appear to play significant role in the pathogenesis of anaemia in chronic disorders. These are: *defective red cell production* and *reduced red cell life-span*.

1. Defective red cell production. Though there is abundance of storage iron in these conditions but the amount of iron in developing erythroid cells in the marrow is subnormal. The mononuclear phagocyte system is hyperplastic and traps all the available free iron due to the activity of iron binding protein, lactoferrin. A defect in the transfer of iron from macrophages to the developing erythroid cells in the marrow leads to reduced availability of iron for haem synthesis despite adequate iron stores, elevating serum ferritin levels. The defect lies in suppression by cytokines at some stage in erythropoiesis e.g. TNF and IFN- β released in bacterial infections and tumours, IL-1 and IFN- γ released in patients of rheumatoid arthritis and autoimmune vasculitis.

2. Reduced red cell life-span. Slightly decreased survival of circulating red cells is also attributed to hyperplastic mononuclear phagocyte system.

Laboratory Findings

The characteristic features of anaemia in these patients uncomplicated by other deficiencies are as under:

i) Haemoglobin. Anaemia is generally mild to moderate. A haemoglobin value of less than 8 g/dl suggests the presence of additional contributory factors.

ii) Blood picture. The type of anaemia in these cases is generally normocytic normochromic but may have slight microcytosis and hypochromia.

iii) Absolute values. Red cell indices indicate that in spite of normocytic normochromic anaemia, MCHC is slightly low.

iv) Reticulocyte count. The reticulocyte count is generally low.

v) Red cell survival. Measurement of erythrocyte survival generally reveals mild to moderate shortening of their life-span.

vi) Bone marrow. Examination of the marrow generally reveals normal erythroid maturation. However, the red cell precursors have reduced stainable iron than normal, while the macrophages in the marrow usually contain increased amount of iron. Cases of chronic infection often have myeloid hyperplasia and increase in plasma cells.

vii) Serum iron and TIBC. Both serum iron and TIBC are characteristically reduced in this group of anaemias (in contrast to iron deficiency where there is reduction in serum iron but no fall in TIBC, see Table 33.6).

viii) Serum ferritin. Serum ferritin levels are increased in these patients.

ix) Other plasma proteins. In addition, certain other plasma proteins called '*phase reactants*' are raised in patients with chronic inflammation, probably under the stimulus of interleukin-1 released by activated macrophages. These proteins include γ -globulin, C3, haptoglobin, α_1 -antitrypsin and fibrinogen. Elevation of these proteins is responsible for raised ESR commonly present in these patients.

MEGALOBLASTIC ANAEMIA

The megaloblastic anaemias are disorders caused by impaired DNA synthesis and are characterised by a distinctive abnormality in the haematopoietic precursors in the bone marrow in which the *maturation of the nucleus is delayed relative to that of the cytoplasm*. Since cell division is slow but cytoplasmic development progresses

normally, the nucleated red cell precursors tend to be larger which Ehrlich in 1880 termed *megaloblasts*. Megaloblasts are both morphologically and functionally abnormal with the result that the mature red cells formed from them and released into the peripheral blood are also abnormal in shape and size, the most prominent abnormality being *macrocytosis*.

The underlying defect for the asynchronous maturation of the nucleus is defective DNA synthesis due to deficiency of vitamin B₁₂ (cobalamin) and/or folic acid (folate). Less common causes are interference with DNA synthesis by congenital or acquired abnormalities of vitamin B₁₂ or folic acid metabolism. Before considering the megaloblastic anaemia, a brief account of vitamin B₁₂ and folic acid metabolism is considered in order.

The salient nutritional aspects and metabolic functions of vitamin B₁₂ and folic acid are summarised in Table 33.8.

Vitamin B₁₂ Metabolism

BIOCHEMISTRY. Vitamin B₁₂ or cobalamin is a complex organometallic compound having a cobalt atom situated within a corrin ring, similar to the structure of porphyrin from which haem is formed. In humans, there are 2 metabolically active forms of cobalamin—methylcobalamin and adenosyl-cobalamin, which act as coenzymes. The therapeutic vitamin B₁₂ preparation is called cyanocobalamin.

SOURCES. The only dietary sources of vitamin B₁₂ are foods of animal protein origin such as kidney, liver, heart, muscle meats, fish, eggs, cheese and milk. In contrast to folate, vegetables contain practically no vitamin B₁₂. Cooking has little effect on its activity. Vitamin B₁₂ is synthesised in the human large bowel by micro-organisms but is not absorbed from this site and, thus, the humans are entirely dependent upon dietary sources. The average daily requirement for vitamin B₁₂ is 2-4 µg.

ABSORPTION. After ingestion, vitamin B₁₂ in food is released and forms a stable complex with gastric R-binder. R-binder is a form of glycoprotein found in various secretions (e.g. saliva, milk, gastric juice, bile), phagocytes and plasma. On entering the duodenum, the vitamin B₁₂-R-binder complex is digested releasing vitamin B₁₂ which then binds to intrinsic factor (IF). The IF is a glycoprotein of molecular weight 50,000 produced by the parietal cells of the stomach and its secretion roughly parallels that of hydrochloric acid. The vitamin B₁₂-IF complex, on reaching the distal ileum, binds to the specific receptors on the mucosal brush border, thereby enabling the vitamin to be absorbed. The IF, therefore, acts as cell-directed carrier protein similar to transferrin. The receptor-bound vitamin B₁₂-IF complex is taken into the ileal mucosal cells where after several hours the IF is destroyed, vitamin B₁₂ released and is transferred to another transport protein, transcobalamin (TC) II. The vitamin B₁₂-TC II complex is finally secreted into the portal circulation from where it is taken by the liver, bone marrow and other cells. There are 2 major vitamin B₁₂ binding proteins—TC I and TC II, and a minor protein TC III. TC I is not essential for vitamin B₁₂ transport but functions primarily as a storage protein while TC III is similar to TC II and binds a small amount of vitamin B₁₂ (Fig. 33.11,B).

TISSUE STORES. Normally, the liver is the principal storage site of vitamin B₁₂ and stores about 2 mg of the vitamin, while other tissues like kidney, heart and brain together store about 2 mg. The body stores of vitamin B₁₂ are adequate for 2-4 years. Major source of loss is via bile and shedding of intestinal epithelial cells. A major part of the excreted vitamin B₁₂ is reabsorbed in the ileum by the IF resulting in enterohepatic circulation.

FUNCTIONS. Vitamin B₁₂ plays an important role in general cell metabolism, particularly essential for normal haematopoiesis and for maintenance of integrity of the nervous system. Vitamin B₁₂ acts as a co-enzyme for 2 main biochemical reactions in the body:

TABLE 33.8: Salient Features of Vitamin B₁₂ and Folate Metabolism.

FEATURE	VITAMIN B ₁₂	FOLATE
1. Main foods	Animal proteins only	Green vegetables, meats
2. Cooking	Little effect	Easily destroyed
3. Daily requirements	2-4 µg	100-200 µg
4. Daily intake	5-30 µg	100-500 µg
5. Site of absorption	Ileum	Duodenum and jejunum
6. Mechanism of absorption	Intrinsic factor	Conversion to methyl-THF
7. Body stores	2-3 mg (enough for 2-4 yrs)	10-12 mg (enough for 4 months)

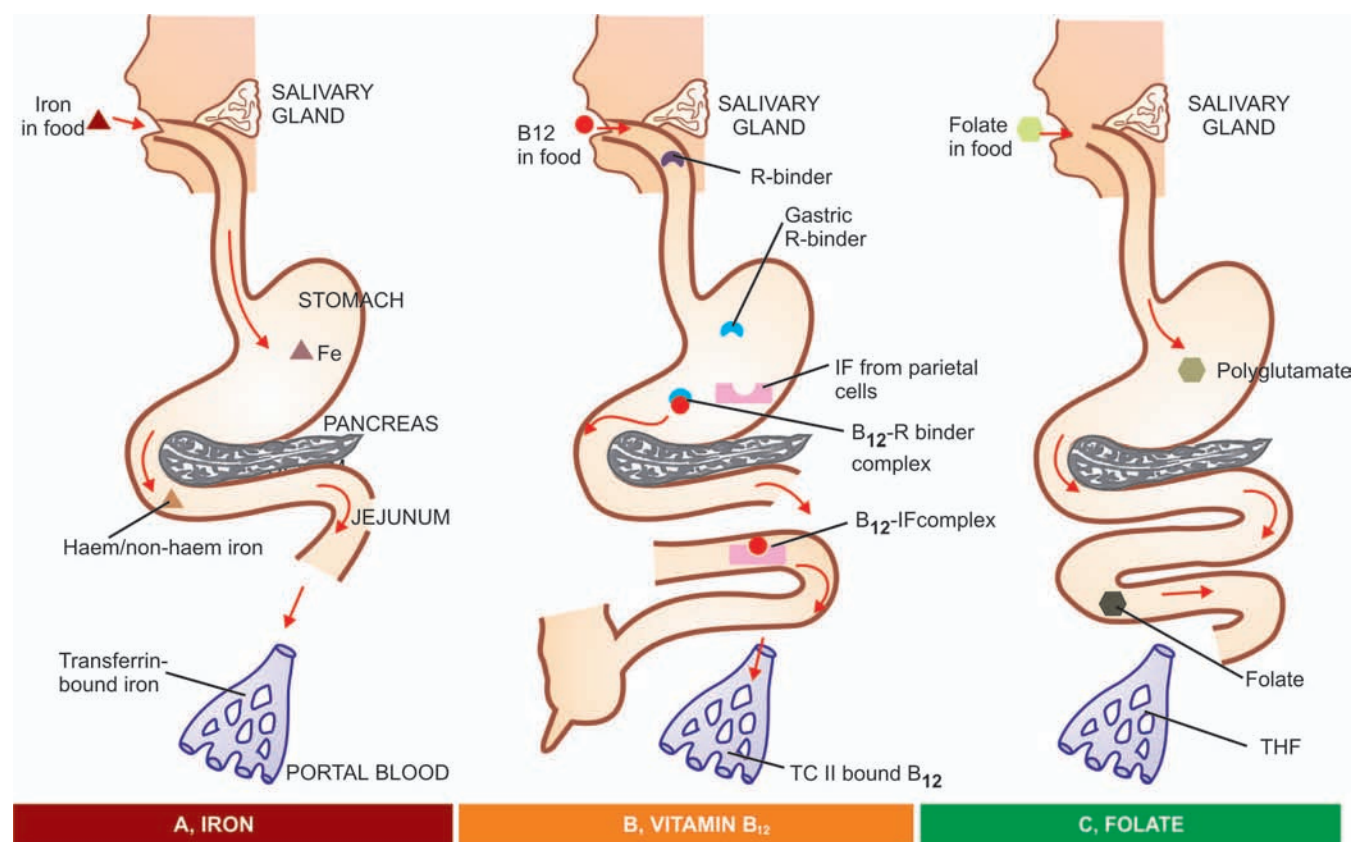
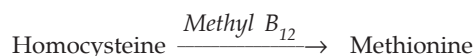


FIGURE 33.11

Contrasting pathways of absorption and transport of iron, vitamin B₁₂ and folic acid.

■ Firstly, as methyl cobalamin (methyl B₁₂) in the methylation of homocysteine to methionine by methyl tetrahydrofolate (THF). The homocysteine-methionine reaction is closely linked to folate metabolism (Fig. 33.12):



When this reaction is impaired, folate metabolism is deranged and results in defective DNA synthesis responsible for megaloblastic maturation.

■ Secondly, as adenosyl cobalamin (adenosyl B₁₂) in propionate metabolism for the conversion of methyl malonyl co-enzyme A to succinyl co-enzyme A:



Lack of adenosyl B₁₂ leads to large increase in the level of methyl malonyl CoA and its precursor, propionyl CoA. This results in synthesis of certain fatty acids which are incorporated into the neuronal lipids. This

biochemical abnormality may contribute to the neurologic complications of vitamin B₁₂ deficiency.

Folate Metabolism

BIOCHEMISTRY. Folate or folic acid, a yellow compound, is a member of water-soluble B complex vitamins with the chemical name of *pteroyl glutamic acid* (PGA). Folic acid does not exist as such in nature but exists as folates in polyglutamate form (conjugated folates). For its metabolic action as co-enzyme, polyglutamates must be reduced to dihydro- and tetrahydrofolate forms.

SOURCES. Folate exists in different plants, bacteria and animal tissues. Its main dietary sources are fresh green leafy vegetables, fruits, liver, kidney, and to a lesser extent, muscle meats, cereals and milk. Folate is labile and is largely destroyed by cooking and canning. Some amount of folate synthesised by bacteria in the human large bowel is not available to the body since its absorption takes place in the small intestine. Thus,

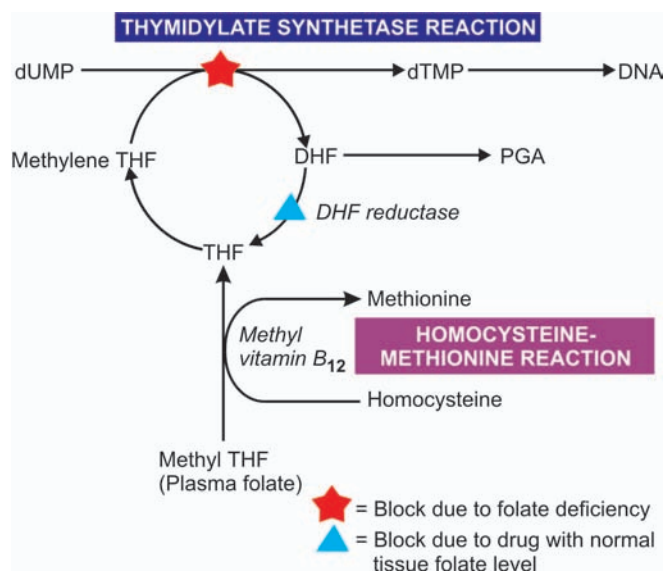


FIGURE 33.12

Biochemical basis of megaloblastic anaemia (THF = tetrahydrofolate; DHF = dihydrofolate; PGA = pteroyl glutamic acid; dUMP = deoxy uridylyte monophosphate; dTMP = deoxy thymidylate monophosphate).

humans are mainly dependent upon diet for its supply. The average daily requirement is 100-200 µg.

ABSORPTION AND TRANSPORT. Folate is normally absorbed from the duodenum and upper jejunum and to a lesser extent, from the lower jejunum and ileum. However, absorption depends upon the form of folate in the diet. Polyglutamate form in the foodstuffs is first cleaved by the enzyme, folate conjugase, in the mucosal cells to mono- and diglutamates which are readily assimilated. Synthetic folic acid preparations in polyglutamate form are also absorbed as rapidly as mono- and diglutamate form because of the absence of natural inhibitors. Mono- and diglutamates undergo further reduction in the mucosal cells to form tetrahydrofolate (THF), a monoglutamate. THF circulates in the plasma as methylated compound, methyl THF, bound to a protein. Once methyl THF is transported into the cell by a carrier protein, it is reconverted to polyglutamate (Fig. 33.11,C).

TISSUE STORES. The liver and red cells are the main storage sites of folate, largely as methyl THF polyglutamate form. The total body stores of folate are about 10-12 mg enough for about 4 months. Normally, folate is lost from the sweat, saliva, urine and faeces.

FUNCTIONS. Folate plays an essential role in cellular metabolism. It acts as a co-enzyme for 2 important biochemical reactions involving transfer of 1-carbon units (viz. methyl and formyl groups) to various other compounds. These reactions are as under:

■ **Thymidylate synthetase reaction.** Formation of deoxy thymidylate monophosphate (dTMP) from its precursor form, deoxy uridylyte monophosphate (dUMP).

■ **Methylation of homocysteine to methionine.** This reaction is linked to vitamin B₁₂ metabolism (Fig. 33.12).

These biochemical reactions are considered in detail below together with biochemical basis of the megaloblastic anaemia.

Biochemical Basis of Megaloblastic Anaemia

The basic biochemical abnormality common to both vitamin B₁₂ and folate deficiency is a block in the DNA synthesis pathway and that there is an inter-relationship between vitamin B₁₂ and folate metabolism in the methylation reaction of homocysteine to methionine (Fig. 33.12).

As stated above, folate as co-enzyme methylene THF, is required for transfer of 1-carbon moieties (e.g. methyl and formyl) to form building blocks in DNA synthesis. These 1-carbon moieties are derived from serine or formiminoglutamic acid (FIGLU). Two of the important folate-dependent (1-carbon transfer) reactions for formation of building blocks in DNA synthesis are as under:

1. Thymidylate synthetase reaction. This reaction involves synthesis of deoxy thymidylate monophosphate (dTMP) from deoxy uridylyte monophosphate (dUMP). The methyl group of dUMP → dTMP reaction is supplied by the co-enzyme, methylene-THF. After the transfer of 1-carbon from methylene-THF, dihydrofolate (DHF) is produced which must be reduced to active THF by the enzyme DHF-reductase before it can participate in further 1-carbon transfer reaction. Drugs like methotrexate (anti-cancer) and pyrimethamine (antimalarial) are inhibitory to the enzyme, DHF-reductase, thereby inhibiting the DNA synthesis.

2. Homocysteine-methionine reaction. Homocysteine is converted into methionine by transfer of a methyl group from methylene-THF. After transfer of 1-carbon from methylene-THF, THF is produced. This reaction requires the presence of vitamin B₁₂ (methyl-B₁₂).

Deficiency of folate from any cause results in reduced supply of the coenzyme, methylene-THF, and thus interferes with the synthesis of DNA. Deficiency of

vitamin B₁₂ traps folate as its transport form, methyl-THF, thereby resulting in reduced formation of the active form, methylene-THF, needed for DNA synthesis. This is referred to as *methyl-folate trap hypothesis*. An alternative hypothesis of inter-relationship of B₁₂ and folate is the *formate-saturation hypothesis*. According to this hypothesis, the active substrate is formyl-THF. Vitamin B₁₂ deficiency results in reduced supply of formate to THF causing reduced generation of the active compound, formyl THF.

Etiology and Classification of Megaloblastic Anaemia

The etiology of megaloblastic anaemia varies in different parts of the world. As outlined in Table 33.9, megaloblastic anaemia is classified into 3 broad groups: vitamin B₁₂ deficiency, folate deficiency, and other causes.

1. VITAMIN B₁₂ DEFICIENCY. In Western countries, the deficiency of vitamin B₁₂ is usually due to pernicious (Addisonian) anaemia. True vegetarians like in India and breast-fed infants have dietary lack of vitamin B₁₂.

TABLE 33.9: Etiologic Classification of Megaloblastic Anaemia.

I. VITAMIN B₁₂ DEFICIENCY

A. Inadequate dietary intake e.g. strict vegetarians, breast-fed infants.

B. Malabsorption

1. *Gastric causes:* pernicious anaemia, gastrectomy, congenital lack of intrinsic factor.
2. *Intestinal causes:* tropical sprue, ileal resection, Crohn's disease, intestinal blind loop syndrome, fish-tapeworm infestation.

II. FOLATE DEFICIENCY

A. Inadequate dietary intake e.g. in alcoholics, teenagers, infants, old age, poverty.

B. Malabsorption e.g. in tropical sprue, coeliac disease, partial gastrectomy, jejunal resection, Crohn's disease.

C. Excess demand

1. *Physiological:* pregnancy, lactation, infancy.
2. *Pathological :* malignancy, increased haematopoiesis, chronic exfoliative skin disorders, tuberculosis, rheumatoid arthritis.

D. Excess urinary folate loss e.g. in active liver disease, congestive heart failure.

III. OTHER CAUSES

A. Impaired metabolism e.g. inhibitors of dihydrofolate (DHF) reductase such as methotrexate and pyrimethamine; alcohol, congenital enzyme deficiencies.

B. Unknown etiology e.g. in Di Guglielmo's syndrome, congenital dyserythropoietic anaemia, refractory megaloblastic anaemia.

Gastrectomy by lack of intrinsic factor, and small intestinal lesions involving distal ileum where absorption of vitamin B₁₂ occurs, may cause deficiency of the vitamin. Deficiency of vitamin B₁₂ takes at least 2 years to develop when the body stores are totally depleted.

2. FOLATE DEFICIENCY. Folate deficiency is more often due to poor dietary intake. Other causes include malabsorption, excess folate utilisation such as in pregnancy and in various disease states, alcoholism, and excess urinary folate loss. Folate deficiency arises more rapidly than vitamin B₁₂ deficiency since the body's stores of folate are relatively low which can last for up to 4 months only.

Patients with tropical sprue are often deficient in both vitamin B₁₂ and folate. Combined deficiency of vitamin B₁₂ and folate may occur from severe deficiency of vitamin B₁₂ because of the biochemical inter-relationship with folate metabolism.

3. OTHER CAUSES. In addition to deficiency of vitamin B₁₂ and folate, megaloblastic anaemias may occasionally be induced by other factors unrelated to vitamin deficiency. These include many drugs which interfere with DNA synthesis, acquired defects of haematopoietic stem cells, and rarely, congenital enzyme deficiencies.

Clinical Features

Deficiency of vitamin B₁₂ and folate may cause the following clinical manifestations which may be present singly or in combination and in varying severity:

1. Anaemia. Macrocytic megaloblastic anaemia is the cardinal feature of deficiency of vitamin B₁₂ and/or folate. The onset of anaemia is usually insidious and gradually progressive.

2. Glossitis. Typically, the patient has a smooth, beefy, red tongue.

3. Neurologic manifestations. Vitamin B₁₂ deficiency, particularly in patients of pernicious anaemia, is associated with significant neurological manifestations in the form of subacute combined, degeneration of the spinal cord and peripheral neuropathy, while folate deficiency may occasionally develop neuropathy only. The underlying pathologic process consists of demyelination of the peripheral nerves, the spinal cord and the cerebrum. Signs and symptoms include numbness, paraesthesia, weakness, ataxia, poor finger coordination and diminished reflexes.

4. Others. In addition to the cardinal features mentioned above, patients may have various other symp-

toms. These include: mild jaundice, angular stomatitis, purpura, melanin pigmentation, symptoms of malabsorption, weight loss and anorexia.

Laboratory Findings

The investigations of a suspected case of megaloblastic anaemia are aimed at 2 aspects:

A. *General laboratory investigations of anaemia* which include blood picture, red cell indices, bone marrow findings, and biochemical tests.

B. *Special tests* to establish the cause of megaloblastic anaemia as to know whether it is due to deficiency of vitamin B₁₂ or folate.

Based on these principles, the following plan of investigations is followed:

A. General Laboratory Findings

These are as under (Fig. 33.13):

1. BLOOD PICTURE AND RED CELL INDICES.

The estimation of haemoglobin, examination of a blood film and evaluation of absolute values are essential preliminary investigations.

i) **Haemoglobin.** The haemoglobin estimation reveals values below the normal range. The fall in haemoglobin concentration may be of a variable degree.

ii) **Red cells.** The red blood cell morphology in a blood film shows the characteristic macrocytosis.

However, *macrocytosis* can also be seen in several other disorders such as: haemolysis, liver disease, alcoholism, hypothyroidism, aplastic anaemia, myeloproliferative disorders and reticulocytosis. In addition, the blood smear demonstrates marked anisocytosis, poikilocytosis and presence of macro-ovalocytes. Basophilic stippling and occasional normoblast may also be seen (**COLOUR PLATE X: CL 38**).

iii) **Reticulocyte count.** The reticulocyte count is generally low to normal in untreated cases.

iv) **Absolute values.** The red cell indices reveal an elevated MCV (above 120 fl) proportionate to the severity of macrocytosis, elevated MCH (above 50 pg) and normal or reduced MCHC.

v) **Leucocytes.** The total white blood cell count may be reduced. Presence of characteristic hypersegmented neutrophils (having more than 5 nuclear lobes) in the blood film should raise the suspicion of megaloblastic anaemia. An occasional myelocyte may also be seen.

vi) **Platelets.** Platelet count may be moderately reduced in severely anaemic patients. Bizarre forms of platelets may be seen.

2. **BONE MARROW FINDINGS.** The bone marrow examination is very helpful in the diagnosis of megaloblastic anaemia. Significant findings of marrow examination are as under:

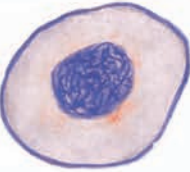
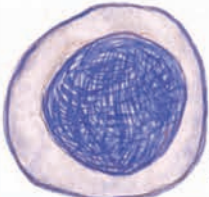
LABORATORY FINDINGS		NORMAL	MEGALOBLASTIC ANAEMIA
BLOOD	RED CELL MORPHOLOGY	 Normal red cell	 Macrocytic red cell
	RED CELL INDICES	MCV, MCH, MCHC all normal	MCV ↑, MCH ↑, MCHC Normal or ↓
BONE MARROW	MARROW ERYTHROPOIESIS	 Normoblastic	 Megaloblastic
	MARROW IRON STORES	Normal	Increased

FIGURE 33.13

General laboratory findings in megaloblastic anaemia.

i) **Marrow cellularity.** The marrow is hypercellular with a decreased myeloid-erythroid ratio.

ii) **Erythropoiesis.** The erythroid hyperplasia is due to characteristic megaloblastic erythropoiesis. *Megaloblasts* are abnormal, large, nucleated erythroid precursors, having nuclear-cytoplasmic asynchrony i.e. the nuclei are less mature than the development of cytoplasm. The nuclei are large, having fine, reticular and open chromatin that stains lightly, while the haemoglobinisation of the cytoplasm proceeds normally or at a faster rate i.e. *nuclear maturation lags behind that of cytoplasm* (compared from iron deficiency anaemia in which cytoplasmic maturation lags behind, page 451). Megaloblasts with abnormal mitoses may be seen. Features of ineffective erythropoiesis such as presence of degenerated erythroid precursors may be present.

iii) **Other cells.** Granulocyte precursors are also affected to some extent. Giant forms of metamyelocytes and band cells may be present in the marrow. Megakaryocytes are usually present in normal number but may occasionally be decreased and show abnormal morphology such as hypersegmented nuclei and agranular cytoplasm.

iv) **Marrow iron.** Prussian blue staining for iron in the marrow shows an increase in the number and size of iron granules in the erythroid precursors. Ring sideroblasts are, however, rare. Iron in the reticulum cells is increased.

3. BIOCHEMICAL FINDINGS. In addition to the general blood and marrow investigations and specific tests to determine the cause of deficiency (described below), the following biochemical abnormalities are observed in cases of megaloblastic anaemia:

i) There is rise in *serum unconjugated bilirubin and LDH* as a result of ineffective erythropoiesis causing marrow cell break down.

ii) The *serum iron and ferritin* may be normal or elevated.

B. Special Tests for Cause of Specific Deficiency

In evaluating a patient of megaloblastic anaemia, it is important to determine the specific vitamin deficiency by assay of vitamin B₁₂ and folate.

TESTS FOR VITAMIN B₁₂ DEFICIENCY. The normal range of vitamin B₁₂ in serum is 200-900 pg/ml (or 200-900 ng/L). Values less than 100 pg/ml indicate clinically deficient stage. There are 3 types

of tests to establish the vitamin B₁₂ deficiency: serum vitamin B₁₂ assay, radioisotope absorption test and serum enzyme levels.

1. SERUM VITAMIN B₁₂ ASSAY. Assay of vitamin B₁₂ blood can be done by 2 methods—microbiologic assay and radioassay.

i) **Microbiological assay.** This test is based on the principle that the serum sample to be assayed is added to a medium containing all other essential growth factors required for a vitamin B₁₂-dependent microorganism. The medium alongwith microorganism is incubated and the amount of vitamin B₁₂ determined turbidimetrically which is then compared with the growth produced by a known amount of vitamin B₁₂. Several organisms have been used for this test such as *Euglena gracilis*, *Lactobacillus leichmannii*, *Escherichia coli* and *Ochromonas malhamensis*. *E. gracilis* is, however, considered more sensitive and accurate. The addition of antibiotics to the test interferes with the growth and yields false low result.

ii) **Radioassay.** Assays of serum B₁₂ by radioisotope dilution (RID) and radioimmunoassay (RIA) have been developed. These tests are more sensitive and have the advantage over microbiologic assays in that they are simpler and more rapid, and the results are unaffected by antibiotics and other drugs which may affect the living organisms.

2. SCHILLING TEST (RADIOISOTOPE ABSORPTION TEST). Schilling test is done to detect vitamin B₁₂ deficiency as well as to distinguish and detect lack of IF and malabsorption. The results of test also depend upon good renal function and proper urinary collection. The test is performed in 3 stages as under:

Stage I: Without IF. Oral dose of 0.5-2 µg of radioactively labelled vitamin B₁₂ ('hot' B₁₂) is administered orally. After 2 hours, a large dose (4 mg) of unlabelled vitamin B₁₂ ('cold' B₁₂) is given parenterally. The 'cold' B₁₂ will saturate the serum as well as the tissue binding sites.

■ In normal individuals, more than 7% of 1 µg of oral dose of 'hot' B₁₂ is excreted in 24-hour urinary sample.

■ Patients with IF deficiency excrete lower quantity of 'hot' B₁₂ which is further confirmed by repeating the test as in stage II given below.

Stage II: With IF. If the 24-hour urinary excretion of 'hot' B₁₂ is low, the test is repeated using the same

procedure as in stage I but in addition high oral dose of IF is administered along with 'hot' B₁₂.

■ If the 24-hour urinary excretion of 'hot' B₁₂ is now normal, the low value in first stage of the test was due to IF deficiency.

■ Patients with pernicious anaemia have abnormal test even after treatment with vitamin B₁₂ due to IF deficiency. However, abnormal 24-hour urinary excretion of 'hot' B₁₂ is further investigated in stage III for a cause in intestinal malabsorption of 'hot' B₁₂.

Stage III: Test for malabsorption of vitamin B₁₂. Some patients absorb vitamin B₁₂ in water as was stipulated in the original Schilling test. Currently, modified Schilling test employs the use of protein-bound vitamin B₁₂. In conditions causing malabsorption, the test is repeated after a course of treatment with antibiotics or anti-inflammatory drugs.

3. SERUM ENZYME LEVELS. Besides Schilling test, another way of distinguishing whether megaloblastic anaemia is due to cobalamine or folate is by serum determination of methylmalonic acid and homocysteine. Both are elevated in cobalamine deficiency, while in folate deficiency there is only elevation of homocysteine and not of methylmalonic acid.

TESTS FOR FOLATE DEFICIENCY. The normal range of serum folate is 6-12 ng/ml (6-12 µg/L). Values of 4 ng/ml or less are generally considered to be diagnostic of folate deficiency. Measurement of *formiminoglutamic acid (FIGLU) urinary excretion* after histidine load was used formerly for assessing folate status but it is less specific and less sensitive than the serum assays. Currently, there are 3 tests used to detect folate deficiency—urinary excretion of FIGLU, serum and red cell folate assay.

1. URINARY EXCRETION OF FIGLU. Folic acid is required for conversion of formiminoglutamic acid (FIGLU) to glutamic acid in the catabolism of histidine. Thus, on oral administration of histidine, urinary excretion of FIGLU is increased if folate deficiency is present.

2. SERUM FOLATE ASSAY. The folate in serum can be estimated by 2 methods—microbiological assay and radioassay.

i) Microbiological assay. This test is based on the principle that the serum folate acid activity is mainly due to the presence of a folic acid co-enzyme, 5-methyl THF, and that this compound is required

for growth of the microorganism, *Lactobacillus casei*. The growth of *L. casei* is inhibited by addition of antibiotics.

ii) Radioassay. The principle and method of radioassay by radioisotope dilution (RID) test are similar to that for serum B₁₂ assay. The test employs labelled pteroylglutamic acid or methyl-THF. Commercial kits are available which permit simultaneous assay of both vitamin B₁₂ and folate.

3. RED CELL FOLATE ASSAY. Red cells contain 20-50 times more folate than the serum and that red cell folate assay is more reliable indicator of tissue stores of folate than serum folate assay. Both microbiological and radioassay methods can be used for estimation of red cell folate. Red cell folate values are decreased in patients with megaloblastic anaemia as well as in patients with pernicious anaemia.

Treatment

Most cases of megaloblastic anaemia need therapy with appropriate vitamin. This includes: hydroxycobalamin as intramuscular injection 1000 µg for 3 weeks and oral folic acid 5 mg tablets daily for 4 months. Severely-anaemic patients in whom a definite deficiency of either vitamin cannot be established with certainty are treated with both vitamins concurrently. Blood transfusion should be avoided since it may cause circulatory overload. Packed cells may, however, be infused slowly.

Treatment of megaloblastic anaemia is quite gratifying. Marrow begins to revert back to normal morphology within a few hours of initiating treatment and becomes normoblastic within 48 hours of start of treatment. Reticulocytosis appears within 4-5 days after therapy is started and peaks at day 7. Haemoglobin should rise by 2-3g/dl each fortnight. The peripheral neuropathy may show some improvement but subacute combined degeneration of the spinal cord is irreversible.

PERNICIOUS ANAEMIA

Pernicious anaemia (PA) was first described by Addison in 1855 as a chronic disorder of middle-aged and elderly individual of either sex in which intrinsic factor secretion ceases owing to atrophy of the gastric mucosa. The condition is, therefore, also termed Addisonian megaloblastic anaemia. The average age at presentation is 60 years but rarely it can be seen in children under 10 years of age (juvenile pernicious anaemia). PA is seen most frequently in individuals of northern European descent and American blacks and is uncommon in South Europeans and Orientals.

Pathogenesis

There is evidence to suggest that the atrophy of gastric mucosa in PA is caused by an autoimmune reaction against gastric parietal cells. The evidences in support of immunological abnormalities in pernicious anaemia are as under:

1. The incidence of PA is high in patients with *other autoimmune diseases* such as Graves' disease, myxoedema, thyroiditis, vitiligo, diabetes and idiopathic adrenocortical insufficiency.
2. Patients with PA have abnormal *circulating auto-antibodies* such as anti-parietal cell antibody (90% cases) and anti-intrinsic factor antibody (50% cases).
3. *Relatives* of patients with PA have an increased incidence of the disease or increased presence of auto-antibodies.
4. *Corticosteroids* have been reported to be beneficial in curing the disease both pathologically and clinically.
5. PA is more common in patients with *agammaglobulinaemia* supporting the role of cellular immune system in destruction of parietal cells.

PATHOLOGIC CHANGES The most characteristic pathologic finding in PA is gastric atrophy affecting the acid- and pepsin-secreting portion of the stomach and sparing the antrum. Gastric epithelium may show cellular atypia. About 2-3% cases of PA develop carcinoma of the stomach. Other pathologic changes are secondary to vitamin B₁₂ deficiency and include megaloblastoid alterations in the gastric and intestinal epithelium and neurologic abnormalities such as peripheral neuropathy and spinal cord damage.

Clinical Features

The disease has insidious onset and progresses slowly. The clinical manifestations are mainly due to vitamin B₁₂ deficiency. These include: anaemia, glossitis, neurological abnormalities (neuropathy, subacute combined degeneration of the spinal cord, retrobulbar neuritis), gastrointestinal manifestations (diarrhoea, anorexia, weight loss, dyspepsia), hepatosplenomegaly, congestive heart failure and haemorrhagic manifestations.

Laboratory Findings

Laboratory examination of a case of PA reveals the following abnormalities:

1. *Hypergastrinaemia*
2. *Pentagastrinaemia*

3. *Haematologic findings* in blood and bone marrow are similar to those seen in megaloblastic anaemia discussed above.

4. *Biochemical alterations* reveal rise in serum bilirubin, LDH, haptoglobin, ferritin and iron. Serum folate is usually normal but red cell folate is almost always reduced.

5. *Schilling test* is abnormal due to IF deficiency as observed by low 24-hour urinary excretion of labelled vitamin B₁₂ (page 460).

6. *Chromosomal abnormalities* are frequently present in bone marrow cells which disappear after therapy.

Treatment

Patients of PA are treated with vitamin B₁₂ in the following way:

1. Replacement therapy with vitamin B₁₂.
2. Symptomatic and supportive therapy such as physiotherapy for neurologic deficits and occasionally blood transfusion.

TABLE 33.10: Classification of Haemolytic Anaemias.

I. ACQUIRED (EXTRACORPUSCULAR)

A. Antibody: Immunohaemolytic anaemias

1. Autoimmune haemolytic anaemia (AIHA)
 - i) Warm antibody AIHA
 - ii) Cold antibody AIHA
2. Drug-induced immunohaemolytic anaemia
3. Isoimmune haemolytic anaemia (page 516)

B. Mechanical trauma: Microangiopathic haemolytic anaemia

C. Direct toxic effects: Malaria, bacterial infection and other agents

D. Acquired red cell membrane abnormalities: paroxysmal nocturnal haemoglobinuria (PNH)

E. Splenomegaly

II. HEREDITARY (INTRACORPUSCULAR)

A. Abnormalities of red cell membrane

1. Hereditary spherocytosis
2. Hereditary elliptocytosis (hereditary ovalocytosis)
3. Hereditary stomatocytosis

B. Disorders of red cell interior

1. *Red cell enzyme defects*
 - i) Defects in the hexose monophosphate shunt: G6PD deficiency
 - ii) Defects in the Embden-Meyerhof (or glycolytic) pathway: pyruvate kinase deficiency
2. *Disorders of haemoglobin*
 - i) Structurally abnormal haemoglobins (haemoglobinopathies): sickle syndromes, other haemoglobinopathies
 - ii) Reduced globin chain synthesis: thalassaemias

3. Follow-up for early detection of cancer of the stomach.

Most of the abnormalities due to vitamin B₁₂ deficiency can be corrected except the irreversible damage to the spinal cord. Corticosteroid therapy can improve the gastric lesion with a return of acid secretion but the higher incidence of gastric polyps and cancer of the stomach in these patients can only be detected by frequent follow-up.

HAEMOLYTIC ANAEMIAS

Definition and Classification

Haemolytic anaemias are defined as anaemias resulting from an increase in the rate of red cell destruction. Normally, effete red cells undergo lysis at the end of their life-span of 90-120 days within the cells of reticulo-endothelial (RE) system in the spleen and elsewhere (extravascular haemolysis), and haemoglobin is not liberated into the plasma in appreciable amounts. The red cell life-span is shortened in haemolytic anaemia i.e. there is accelerated haemolysis. However, shortening of red cell life-span does not necessarily result in anaemia. In fact, compensatory bone marrow hyperplasia may cause 6-8-fold increase in red cell production without causing anaemia to the patient, so-called *compensated haemolytic disease*.

The premature destruction of red cells in haemolytic anaemia may occur by 2 mechanisms:

■ **Firstly**, the red cells undergo lysis in the circulation and release their contents into plasma (*intravascular haemolysis*). In these cases the plasma haemoglobin rises substantially and part of it may be excreted in the urine (*haemoglobinuria*).

■ **Secondly**, the red cells are taken up by cells of the RE system where they are destroyed and digested (*extravascular haemolysis*). In extravascular haemolysis, plasma haemoglobin level is, therefore, barely raised.

Extravascular haemolysis is more common than the former. One or more factors may be involved in the pathogenesis of various haemolytic anaemias.

Haemolytic anaemias are broadly classified into 2 main categories:

I. *Acquired haemolytic anaemias* caused by a variety of extrinsic environmental factors (*extracorporeal*).

II. *Hereditary haemolytic anaemias* are usually the result of intrinsic red cell defects (*intracorporeal*).

A simplified classification based on these mechanisms is given in Table 33.10 and diagrammatically represented in Fig. 33.14.

Features of Haemolysis

A number of clinical and laboratory features are shared by various types of haemolytic anaemias. These are briefly described below:

GENERAL CLINICAL FEATURES. Some of the general clinical features common to most congenital and acquired haemolytic anaemias are as under:

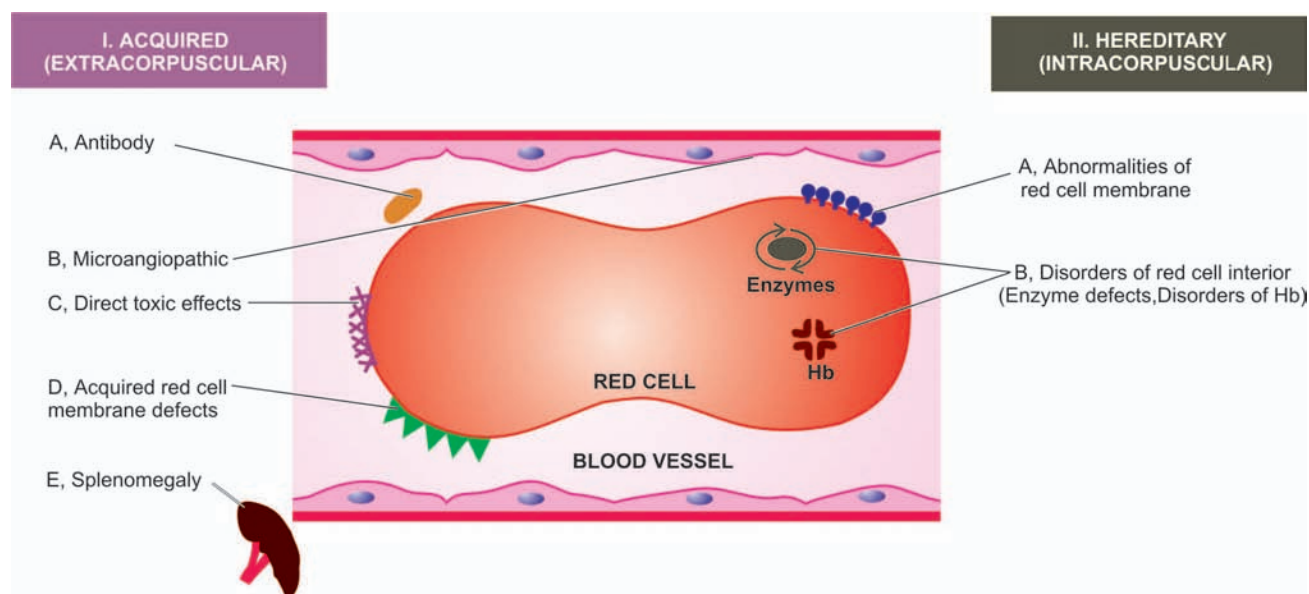


FIGURE 33.14

Diagrammatic representation of classification of haemolytic anaemias based on principal mechanisms of haemolysis.

1. Presence of pallor of mucous membranes.
2. Positive family history with life-long anaemia in patients with congenital haemolytic anaemia.
3. Mild fluctuating jaundice due to unconjugated hyperbilirubinaemia.
4. Urine turns dark on standing due to excess of urobilinogen in urine.
5. Splenomegaly is found in most chronic haemolytic anaemias, both congenital and acquired.
6. Pigment gallstones are found in some cases.

LABORATORY EVALUATION OF HAEMOLYSIS.

The pathways by which haemoglobin derived from effete red cells is metabolised is already discussed on page 441. The investigations of a patient suspected to have haemolytic anaemia should provide answers to 3 vital questions:

1. Is there evidence of haemolysis?
2. What is the type of haemolytic mechanism?
3. What is the precise diagnosis?

And, if facilities are available, certain tests of scientific nature which are not normally required for making the diagnosis or predicting prognosis, may be carried out.

The laboratory findings are conveniently divided into the following 3 groups:

I. Tests of increased red cell breakdown. These include the following:

1. *Serum bilirubin*—unconjugated (indirect) bilirubin is raised.
2. *Urine urobilinogen* is raised but there is no bilirubinuria.
3. *Faecal stercobilinogen* is raised.
4. *Serum haptoglobin* (α -globulin binding protein) is reduced or absent.
5. *Plasma lactic dehydrogenase* is raised.

6. *Evidences of intravascular haemolysis* in the form of haemoglobinaemia, haemoglobinuria, methaemoglobinaemia and haemosiderinuria.

II. Tests of increased red cell production. These are as under:

1. *Reticulocyte count* reveals reticulocytosis which is generally early and is hence most useful initial test of marrow erythroid hyperplasia.
2. *Routine blood film* shows macrocytosis, polychromasia and presence of normoblasts.
3. *Bone marrow* shows erythroid hyperplasia with usually raised iron stores.
4. *X-ray of bones* shows evidence of expansion of marrow space, especially in tubular bones and skull.

III. Tests of damage to red cells. These include the following:

1. *Routine blood film* shows a variety of abnormal morphological appearances of red cells described on page 443 and illustrated in Fig. 13.9 already. A summary of contribution of morphology of RBCs in arriving at the diagnosis of haemolytic anaemia and its cause is given in Table 33.11.
2. *Osmotic fragility* is increased.
3. *Autohaemolysis test* with or without addition of glucose.
4. *Coombs' antiglobulin test*.
5. *Electrophoresis* for abnormal haemoglobins.
6. *Estimation of HbA₂*.
7. *Estimation of HbF*.
8. *Tests for sickling*.
9. *Screening test for G6PD deficiency* and other enzymes (e.g. Heinz bodies test).

IV. Tests for shortened red cell life-span. A shortened red cell survival is best tested by ⁵¹Cr labelling method.

TABLE 33.11: Red Cell Morphologic Features in Various Types of Haemolytic Anaemias.

FEATURE	ETIOLOGY	TYPE OF HAEMOLYTIC ANAEMIA
1. <i>Spherocytes</i>	Loss of spectrin from membrane	Hereditary spherocytosis AIHA
2. <i>Target cells (Leptocytes)</i>	Increased ratio of surface area: volume	Thalassaemias Liver disease HbS disease HbC disease
3. <i>Schistocytes</i>	Traumatic damage to red cell membrane	Microangiopathy
4. <i>Sickle cells</i>	Polymerisation of HbS	Sickle syndromes
5. <i>Acanthocytes (Spur cells)</i>	Abnormality in membrane lipids	Severe liver disease
6. <i>Heinz bodies</i>	Precipitated Hb	Unstable Hb

Normal RBC life-span of 120 days is shortened to 20-40 days in moderate haemolysis and to 5-20 days in severe haemolysis.

I. ACQUIRED (EXTRACORPUSCULAR) HAEMOLYTIC ANAEMIAS

Acquired haemolytic anaemias are caused by a variety of extrinsic factors, namely: antibody (immunohaemolytic anaemia), mechanical factors (microangiopathic haemolytic anaemia), direct toxic effect (in malaria, clostridial infection etc), splenomegaly, and certain acquired membrane abnormalities (paroxysmal nocturnal haemoglobinuria). These are discussed below:

A. IMMUNOHAEMOLYTIC ANAEMIAS

Immunohaemolytic anaemias are a group of anaemias occurring due to antibody production by the body against its own red cells. Immune haemolysis in these cases may be induced by one of the following three types of antibodies:

1. *Autoimmune haemolytic anaemia (AIHA)* characterised by formation of autoantibodies against patient's own red cells. Depending upon the reactivity of autoantibody, AIHA is further divided into 2 types:
 - i) 'Warm' antibody AIHA in which the autoantibodies are reactive at body temperature (37°C).
 - ii) 'Cold' antibody AIHA in which the autoantibodies react better with patient's own red cells at 4°C.
2. *Drug-induced immunohaemolytic anaemia.*
3. *Isoimmune haemolytic anaemia* in which the antibodies are acquired by blood transfusions, pregnancies and haemolytic disease of the newborn.

An important diagnostic tool in all cases of immunohaemolytic anaemias is Coombs' antiglobulin test for detection of incomplete Rh-antibodies in saline directly (direct Coombs') or after addition of albumin (indirect Coombs').

Autoimmune Haemolytic Anaemia (AIHA)

'WARM' ANTIBODY AIHA

PATHOGENESIS. Warm antibodies reactive at body temperature and coating the red cells are generally IgG class antibodies and occasionally they are IgA. Little is known about the origin of these acquired red blood cell antibodies in AIHA but the mechanism of destruction of red cells coated with IgG is better understood. Human red cells coated with IgG antibodies are bound to the surface of RE cells, especially splenic macrophages. A part of the coated cell membrane is lost resulting in

spherical transformation of the red cells (acquired spherocytosis). Red cells coated with IgG alongwith C3 on the surface further promote this red cell-leucocyte interaction, accounting for more severe haemolysis. The spleen is particularly efficient in trapping red cells coated with IgG antibodies. It is, thus, the major site of red cell destruction in warm antibody AIHA.

CLINICAL FEATURES. Warm antibody AIHA may occur at any age and in either sex. The disease may occur without any apparent cause (idiopathic) but about a quarter of patients develop this disorder as a complication of an underlying disease affecting the immune system, especially SLE, chronic lymphocytic leukaemia, lymphomas and certain drugs such as methyl DOPA, penicillin etc (Table 33.12).

The disease tends to have remissions and relapses. The usual clinical features are:

1. Chronic anaemia of varying severity with remissions and relapses.
2. Splenomegaly.
3. Occasionally hyperbilirubinaemia.

Treatment of these cases consists of removal of the cause whenever present, corticosteroid therapy, and in severe cases blood transfusions. Splenectomy is the second line of therapy in this disorder.

LABORATORY FINDINGS. The haematological and biochemical findings in such cases are as under:

1. Mild to moderate chronic anaemia.
2. Reticulocytosis.
3. Prominent spherocytosis in the peripheral blood film.
4. Positive direct Coombs' (antiglobulin) test for presence of warm antibodies on the red cell, best detected at 37°C.

TABLE 33.12: Conditions Predisposing to Autoimmune Haemolytic Anaemia (AIHA).

A. Warm antibody AIHA	
1.	Idiopathic (primary)
2.	Lymphomas-leukaemias e.g. non-Hodgkin's lymphoma, CLL, Hodgkin's disease.
3.	Collagen vascular diseases e.g SLE
4.	Drugs e.g. methyl dopa, penicillin, quinidine group
5.	Post-viral
B. Cold antibody AIHA	
1.	Cold agglutinin disease
a)	Acute: <i>Mycoplasma</i> infection, infectious mononucleosis
b)	Chronic: Idiopathic, lymphomas
2.	PCH (<i>Mycoplasma</i> infection, viral flu, measles, mumps, syphilis)

5. A positive indirect Coombs' (antiglobulin) test at 37°C may indicate presence of large quantities of warm antibodies in the serum.
6. Unconjugated (indirect) hyperbilirubinaemia.
7. Co-existent immune thrombocytopenia along with occasional venous thrombosis may be present (termed Evans' syndrome).
8. In more severe cases, haemoglobinaemia and haemoglobinuria may be present.

'COLD' ANTIBODY AIHA

PATHOGENESIS. Antibodies which are reactive in the cold (4°C) may induce haemolysis under 2 conditions: cold agglutinin disease and paroxysmal cold haemoglobinuria.

1. Cold agglutinin disease. In cold agglutinin disease, the antibodies are IgM type which bind to the red cells best at 4°C. These cold antibodies are usually directed against the *I* antigen on the red cell surface. Agglutination of red blood cells by IgM cold agglutinins is most profound at very low temperature but upon warming to 37°C or above, disagglutination occurs quickly. Haemolytic effect is mediated through fixation of C3 to the red blood cell surface and not by agglutination alone. Most cold agglutinins affect juvenile red blood cells.

The etiology of cold antibody remains unknown. It is seen in the course of certain infections (e.g. *Mycoplasma* pneumonia, infectious mononucleosis) and in lymphomas.

2. Paroxysmal cold haemoglobinuria (PCH). In PCH, the cold antibody is an IgG antibody (Donath-Landsteiner antibody) which is directed against *P* blood group antigen and brings about complement-mediated haemolysis. Attacks of PCH are precipitated by exposure to cold.

PCH is uncommon and may be seen in association with tertiary syphilis or as a complication of certain infections such as *Mycoplasma* pneumonia, flu, measles and mumps.

CLINICAL FEATURES. The clinical manifestations are due to haemolysis and not due to agglutination. These include:

1. Chronic anaemia which is worsened by exposure to cold.
2. Raynaud's phenomenon.
3. Cyanosis affecting the cold exposed regions such as tips of nose, ears, fingers and toes.

4. Haemoglobinaemia and haemoglobinuria occur on exposure to cold.

Treatment consists of keeping the patient warm and treating the underlying cause.

LABORATORY FINDINGS. The haematologic and biochemical findings are somewhat similar to those found in warm antibody AIHA except the thermal amplitude. These findings are:

1. Chronic anaemia.
2. Low reticulocyte count since young red cells are affected more.
3. Spherocytosis is less marked.
4. Positive direct Coombs' test for detection of C3 on the red cell surface but IgM responsible for C3 coating on red cells is not found.
5. The cold antibody titre is very high at 4°C and very low at 37°C (Donath-Landsteiner test). IgM class cold antibody has specificity for *I* antigen, while the rare IgG class antibody of PCH has *P* blood group antigen specificity.

Drug-induced Immunohaemolytic Anaemia

Drugs may cause immunohaemolytic anaemia by 3 different mechanisms:

1. α -METHYL DOPA TYPE ANTIBODIES. A small proportion of patients receiving α -methyl dopa develops immunohaemolytic anaemia which is identical in every respect to warm antibody AIHA described above.

2. PENICILLIN-INDUCED IMMUNOHAEMOLYSIS. Patients receiving large doses of penicillin or penicillin-type antibiotics develop antibodies against the red blood cell-drug complex which induces haemolysis.

3. INNOCENT BYSTANDER IMMUNOHAEMOLYSIS. Drugs such as quinidine form a complex with plasma proteins to which an antibody forms. This drug-plasma protein-antibody complex may induce lysis of bystander red blood cells or platelets.

In each type of drug-induced immunohaemolytic anaemia, discontinuation of the drug results in gradual disappearance of haemolysis.

Isoimmune Haemolytic Anaemia

Isoimmune haemolytic anaemias are caused by acquiring isoantibodies or alloantibodies by blood transfusions, pregnancies and in haemolytic disease of the newborn. These antibodies produced by one individual are directed against red blood cells of the other. These conditions are considered in Chapter 36.

B. MICROANGIOPATHIC HAEMOLYTIC ANAEMIA

Microangiopathic haemolytic anaemia is caused by abnormalities in the microvasculature. It is generally due to mechanical trauma to the red cells in circulation and is characterised by red cell fragmentation (schistocytosis). There are 3 different ways by which microangiopathic haemolytic anaemia results:

1. EXTERNAL IMPACT. Direct external trauma to red blood cells when they pass through microcirculation, especially over the bony prominences, may cause haemolysis during various activities e.g. in prolonged marchers, joggers, karate players etc. These patients develop haemoglobinaemia, haemoglobinuria (*march haemoglobinuria*), and sometimes myoglobinuria as a result of damage to muscles.

2. CARDIAC HAEMOLYSIS. A small proportion of patients who received prosthetic cardiac valves or artificial grafts develop haemolysis. This has been attributed to direct mechanical trauma to the red cells or shear stress from turbulent blood flow.

3. FIBRIN DEPOSIT IN MICROVASCULATURE. Deposition of fibrin in the microvasculature exposes the red cells to physical obstruction and eventual fragmentation of red cells and trapping of the platelets. Fibrin deposits in the small vessels may occur in the following conditions:

- i) *Abnormalities of the vessel wall* e.g. in hypertension, eclampsia, disseminated cancers, transplant rejection, haemangioma etc.
- ii) *Thrombotic thrombocytopenic purpura.*
- iii) *Haemolytic-uraemic syndrome.*
- iv) *Disseminated intravascular coagulation (DIC)*
- v) *Vasculitis in collagen diseases.*

All these conditions are described in respective sections separately.

C. HAEMOLYTIC ANAEMIA FROM DIRECT TOXIC EFFECTS

Haemolysis may result from direct toxic effects of certain agents. These include the following examples:

1. *Malaria* by direct parasitisation of red cells (black-water fever) (**COLOUR PLATE XI: CL 43**).
2. *Bartonellosis* by direct infection of red cells by the microorganisms.
3. *Septicaemia* with *Clostridium welchii* by damaging the red cells.
4. *Other microorganisms* such as pneumococci, staphylococci and *Escherichia coli*.
5. *Copper* by direct haemolytic effect on red cells in Wilson's disease and patients on haemodialysis.

6. *Lead poisoning* shows basophilic stippling of red blood cells.

7. *Snake and spider bites* cause haemolysis by their venoms.

8. *Extensive burns.*

D. PAROXYSMAL NOCTURNAL HAEMOGLOBINURIA (PNH)

PNH is a rare acquired disorder of red cell membrane in which there is chronic intravascular haemolysis due to undue sensitivity of red blood cells to complement due to defective synthesis of a red cell membrane protein. The defect affects all the cells of myeloid progenitor lineage (RBCs, WBCs, platelets) suggesting a deficient haematopoiesis. The disorder generally presents in adult life.

PATHOGENESIS. PNH is considered as an acquired clonal disease of the cell membrane while normal clone also continues to proliferate. The defect in stem cells is a mutation affecting myeloid progenitor cells that normally is required for the biosynthesis of glycosyl phosphatidyl inositol (GPI) essential for anchoring of the cell; the mutant form of the gene is called PIG-A (phosphatidyl inositol glycan). Thus, as a result of mutation, there is partial or complete deficiency of anchor protein. Out of about 20 such proteins described so far, the lack of two of the proteins—*decay accelerating factor (DAF, CD55)* and a *membrane inhibitor of reactive lysis (MIRL, CD59)*, makes the RBCs sensitive to the lytic effect of complement.

CLINICAL AND LABORATORY FINDINGS.

Clinical and laboratory findings are as under:

- i) Haemolytic anaemia.
- ii) Pancytopenia (mild granulocytopenia and thrombocytopenia frequent).
- iii) Intermittent clinical haemoglobinuria; acute haemolytic episodes occur at night identified by passage of brown urine in the morning.
- iv) Haemosiderinuria very common.
- v) Venous thrombosis common complication.

The presence of inordinate sensitivity of red blood cells, leucocytes and platelets to complement in PNH can be demonstrated *in vitro* by *Ham's test* using red cell lysis at acidic pH or by sucrose haemolysis test.

About 20% cases of PNH may develop myeloproliferative or myelodysplastic disorder and some even develop acute myeloid leukaemia.

E. HAEMOLYTIC ANAEMIA IN SPLENOMEGALY

Haemolytic anaemia is common in splenic enlargement from any cause (Chapter 38). Normally, the spleen acts as a filter and traps the damaged red blood cells, destroys them and the splenic macrophages phagocytose the damaged red cells. A normal spleen poses no risk to normal red blood cells. But splenomegaly exaggerates the damaging effect to which the red cells are exposed. Besides haemolytic anaemia, splenomegaly is usually associated with pancytopenia. Splenectomy or reduction in size of spleen by appropriate therapy relieves the anaemia as well as improves the leucocyte and platelet counts.

II. HEREDITARY (INTRACORPUSCULAR) HAEMOLYTIC ANAEMIA

Hereditary haemolytic anaemias are usually the result of intracorpusecular defects. Accordingly, they are broadly classified into 2 groups (see Table 33.10):

- Those due to hereditary abnormalities of red cell membrane.
- Second are those with hereditary disorders of the interior of the red cells.

A. HEREDITARY ABNORMALITIES OF RED CELL MEMBRANE

The abnormalities of red cell membrane are readily identified on blood film examination. There are 3 important types of inherited red cell membrane defects: hereditary spherocytosis, hereditary elliptocytosis (hereditary ovalocytosis) and hereditary stomatocytosis.

Hereditary Spherocytosis

Hereditary spherocytosis is a common type of hereditary haemolytic anaemia of autosomal dominant inheritance in which the red cell membrane is abnormal.

PATHOGENESIS. The molecular abnormality in hereditary spherocytosis is a defect in proteins which anchor the lipid bilayer to the underlying cytoskeleton. These protein abnormalities are as under and are schematically illustrated in Fig. 33.15:

1. **Spectrin deficiency.** Almost all cases have deficiency in the structural protein of the red cell membrane, spectrin. Spectrin deficiency correlates with the severity of anaemia. Mutation in spectrin by recessive inheritance called α -spectrin causes more severe form of anaemia, while mutation by dominant inheritance forming β -spectrin results in mild form of the disease.

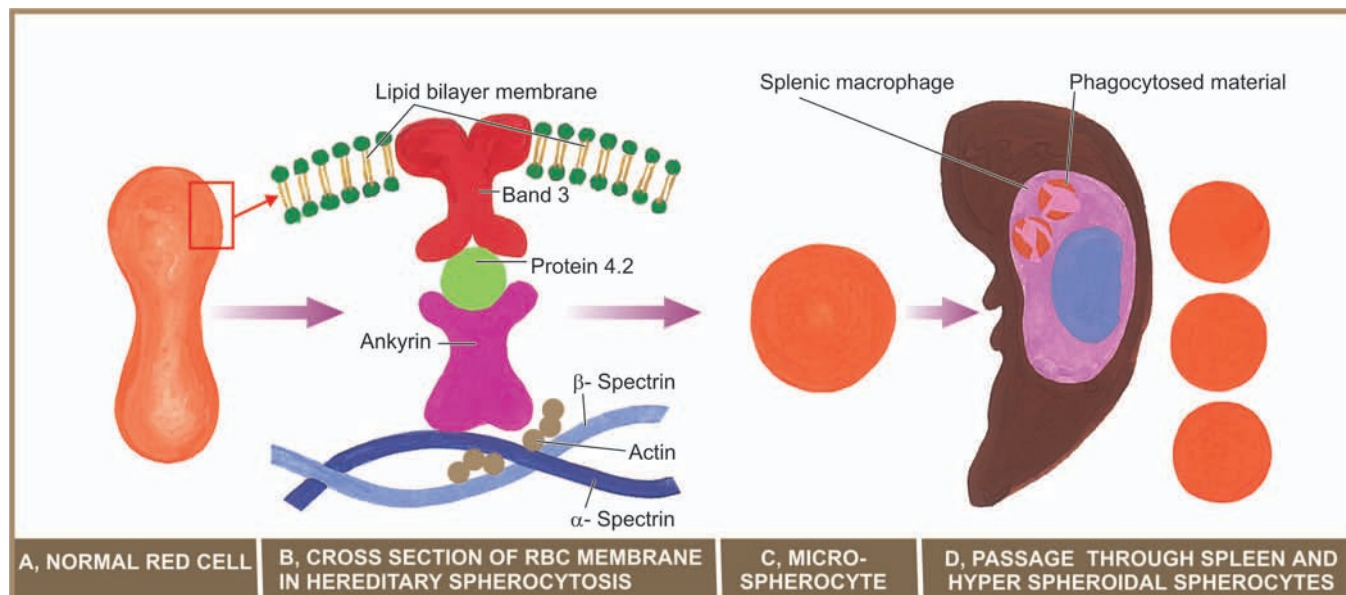


FIGURE 33.15

Diagrammatic representation of pathogenesis of hereditary spherocytosis. A, Normal red cell with biconcave surface and normal size. B, Red cell membrane as seen in cross section in hereditary spherocytosis. Mutations in membrane proteins— α -spectrin, β -spectrin and ankyrin, result in defect in anchoring of lipid bilayer of the membrane to the underlying cytoskeleton. C, This results in spherical contour and small size so as to contain the given volume of haemoglobin in the deformed red cell. D, During passage through the spleen, these rigid spherical cells lose their cell membrane further. This produces a circulating subpopulation of hyperspheroidal spherocytes while splenic macrophages in large numbers phagocytose defective red cells causing splenomegaly.

2. Ankyrin abnormality. About half the cases of hereditary spherocytosis have defect in ankyrin, protein that binds protein 3 and spectrin. Homozygous state with recessive inheritance pattern have severe anaemia while heterozygotes with more common dominant inheritance pattern have milder anaemia.

Inherited mutation in spectrin or ankyrin causes defect in anchoring of lipid bilayer cell membrane. Red cells with such unstable membrane but with normal volume, when released in circulation, lose their membrane further, till they can accommodate the given volume. This results in formation of spheroidal contour and smaller size of red blood cells, termed *microspherocytes*. These deformed red cells are not flexible, unlike normal biconcave red cells. These rigid cells are unable to pass through the spleen, and in the process they lose their surface membrane further. This produces a subpopulation of *hyperspheroidal red cells* in the peripheral blood which are subsequently destroyed in the spleen.

CLINICAL FEATURES. The disorder may be clinically apparent at any age from infancy to old age and has equal sex incidence. The family history may be present. The major clinical features are as under:

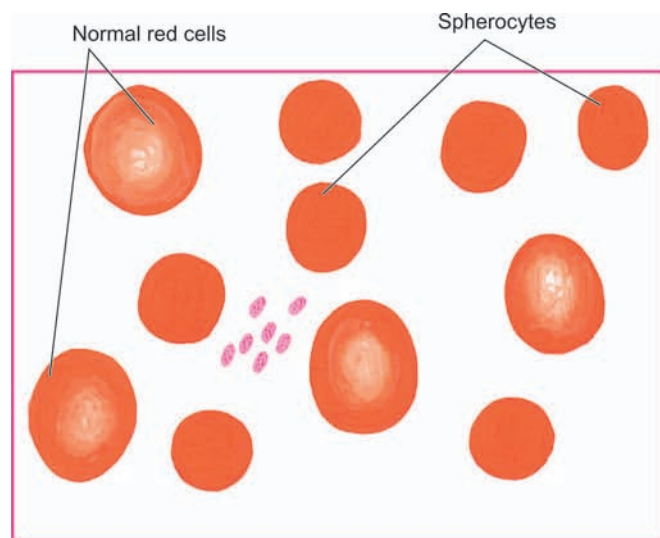
1. *Anaemia* is usually mild to moderate.
2. *Splenomegaly* is a constant feature.
3. *Jaundice* occurs due to increased concentration of unconjugated (indirect) bilirubin in the plasma (also termed congenital haemolytic jaundice).

4. *Pigment gallstones* are frequent due to increased bile pigment production. Splenectomy offers the only reliable mode of treatment.

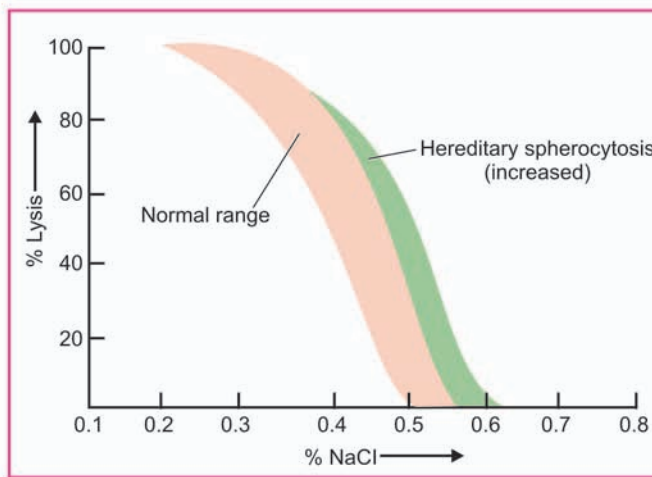
LABORATORY FINDINGS. The usual haematological and biochemical findings are as under:

1. *Anaemia* of mild to moderate degree.
2. *Reticulocytosis*, usually 5-20%.
3. Blood film shows the characteristic abnormality of erythrocytes in the form of *microspherocytes* (Fig. 33.16, A).
4. MCV is usually normal or slightly decreased but MCHC is increased.
5. Osmotic fragility test is helpful in testing the spheroidal nature of red cells which lyse more readily in solutions of low salt concentration i.e. *osmotic fragility is increased* (Fig. 33.16, B).
6. *Autohaemolysis test* is similar to osmotic fragility test after incubation and shows increased spontaneous autohaemolysis (10-15% red cells) as compared to normal red cells (less than 4%). Autohaemolysis is correctable by addition of glucose.
7. *Direct Coombs' (antiglobulin) test* is negative so as to distinguish this condition from acquired spherocytosis of AIHA in which case it is positive.

Spherocytes may also be seen in blood film in acquired immune haemolytic anaemia and following red cell transfusion.



A, BLOOD FILM IN HEREDITARY SPHEROCYTOSIS



B, OSMOTIC FRAGILITY CURVE

FIGURE 33.16

Hereditary spherocytosis. A, Findings in peripheral blood film. B, Osmotic fragility test showing increased fragility.

B. HEREDITARY DISORDERS OF RED CELL INTERIOR

Inherited disorders involving the interior of the red blood cells are classified into 2 groups:

1. **Red cell enzyme defects:** These cause defective red cell metabolism involving 2 pathways (Fig. 33.17):
 - i) *Defects in the hexose monophosphate shunt:* Common example is glucose-6-phosphate dehydrogenase (G6PD) deficiency.
 - ii) *Defects in the Embden-Meyerhof (glycolytic) pathway:* Example is pyruvate kinase (PK) deficiency.
2. **Disorders of haemoglobin:** These are divided into 2 subgroups:
 - i) *Abnormal haemoglobins (haemoglobinopathies):* Examples are sickle syndromes and other haemoglobinopathies.
 - ii) *Reduced globin chain synthesis:* Common examples are various types of thalassaemias.

These disorders are discussed below.

Red Cell Enzyme Defects

G6PD DEFICIENCY

Among the defects in hexose monophosphate shunt, the most common is G6PD deficiency. It affects millions of people throughout the world. The G6PD gene is

located on the X chromosome and its deficiency is, therefore, a sex (X)-linked trait affecting males, while the females are carriers and are asymptomatic. Several variants of G6PD have been described. The normal G6PD variant is designated as type B but blacks have normally A+ (positive) type G6PD variant. The most common and significant clinical variant is A-(negative) type found in black males. Like the HbS gene, the A-type G6PD variant confers protection against malaria. Individuals with A-G6PD variant have shortened red cell life-span but without anaemia. However, these individuals develop haemolytic episodes on exposure to oxidant stress such as viral and bacterial infections, certain drugs (antimalarials, sulfonamides, nitrofurantoin, aspirin, vitamin K), metabolic acidosis and on ingestion of fava beans (favism).

PATHOGENESIS. Normally, the red blood cells are well protected against oxidant stress because of adequate generation of reduced glutathione via the hexose monophosphate shunt (Fig. 33.17). Individuals with inherited deficiency of G6PD, an enzyme required for hexose monophosphate shunt for glucose metabolism, fail to develop adequate levels of reduced glutathione in their red cells. This results in oxidation and precipitation of haemoglobin within the red cells forming Heinz bodies. Besides G6PD deficiency, deficiency of various other enzymes involved in the hexose monophosphate shunt may also infrequently cause clinical problems.

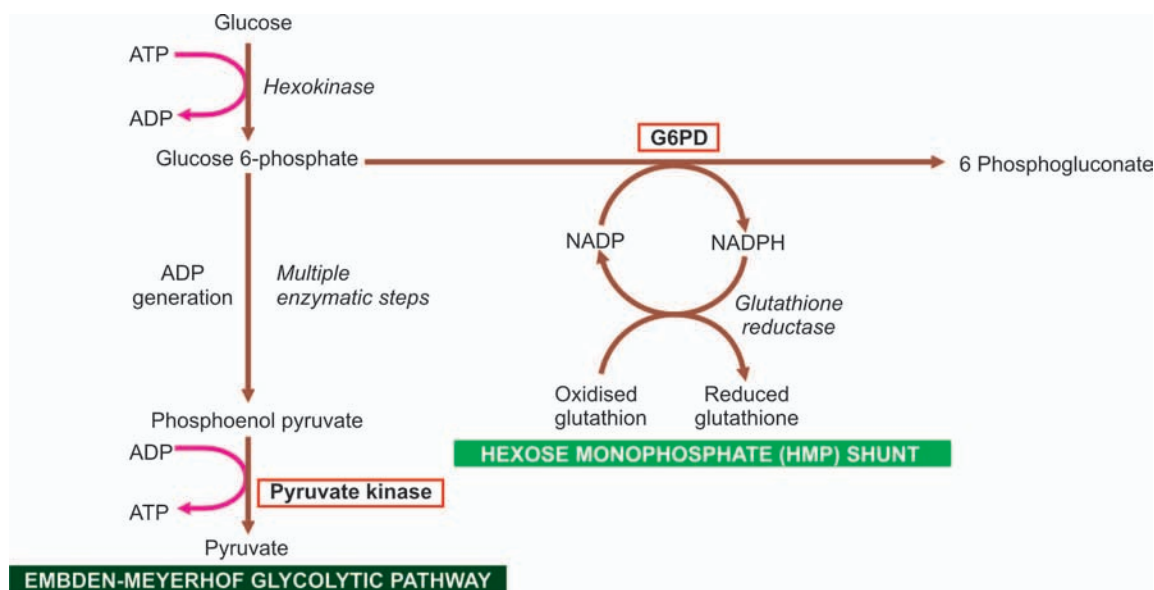


FIGURE 33.17

Abbreviated pathways of anaerobic glycolysis (Embden-Meyerhof) and hexose monophosphate (HMP) shunt in the metabolism of erythrocyte. The two red cell enzyme defects, glucose-6 phosphate dehydrogenase (G6PD) and pyruvate kinase, are shown bold.

CLINICAL FEATURES. The clinical manifestations are those of an acute haemolytic anaemia within hours of exposure to oxidant stress. The haemolysis is, however, self-limiting even if the exposure to the oxidant is continued since it affects the older red cells only. Haemoglobin level may return to normal when the older population of red cells has been destroyed and only younger cells remain. Some patients may have only darkening of the urine from haemoglobinuria but more severely affected ones develop constitutional symptoms including jaundice. Treatment is directed towards the prevention of haemolytic episodes such as stoppage of offending drug. Blood transfusions are rarely indicated.

LABORATORY FINDINGS. These are as under:

1. **During the period of acute haemolysis**, there is rapid fall in haematocrit by 25-30%, features of intravascular haemolysis such as rise in plasma haemoglobin, haemoglobinuria, rise in unconjugated bilirubin and fall in plasma haptoglobin. Formation of Heinz bodies is visualised by means of supravital stains such as crystal violet, also called *Heinz body haemolytic anaemia*. However, Heinz bodies are not seen after the first one or two days since they are removed by the spleen, leading to the formation of 'bite cells' and fragmented red cells.

2. **Between the crises**, the affected patient generally has no anaemia. The red cell survival is, however, shortened.

The diagnosis of G6PD enzyme deficiency is made by one of the screening tests (e.g. methaemoglobin reduction test, fluorescent screening test, ascorbate cyanide screening test), or by direct enzyme assay on red cells.

PK DEFICIENCY

Pyruvate kinase (PK) deficiency is the only significant enzymopathy of the Embden-Meyerhof glycolytic pathway (Fig. 33.17). The disorder is inherited as an autosomal recessive pattern. Heterozygote state is entirely asymptomatic, while the homozygous individual presents during early childhood with anaemia, jaundice and splenomegaly.

LABORATORY FINDINGS: These are:

1. Normocytic and normochromic anaemia.
2. Reticulocytosis.
3. Blood film shows bizarre red cells.
4. Osmotic fragility is usually normal but after incubation it is increased.
5. Autohaemolysis is increased, but unlike hereditary spherocytosis, is not corrected by addition of glucose.

6. Direct specific enzyme assay on red cells is the only method of establishing the diagnosis.

Disorders of Haemoglobin

There are geographic variations in the distribution of various disorders of haemoglobin. These disorders are described below under 2 main headings: abnormal haemoglobins (haemoglobinopathies) and thalassaemias.

ABNORMAL HAEMOGLOBINS (HAEMOGLOBINOPATHIES)

SICKLE SYNDROMES

The most important and widely prevalent type of haemoglobinopathy is due to the presence of sickle haemoglobin (HbS) in the red blood cells. The red cells with HbS develop 'sickling' when they are exposed to low oxygen tension. Sickle syndromes have the highest frequency in black race and in Central Africa where *falciparum* malaria is endemic. Patients with HbS are relatively protected against *falciparum* malaria. Sickle syndromes occur in 3 different forms:

1. *As heterozygous state* for HbS: sickle cell trait (AS).
2. *As homozygous state* for HbS: sickle cell anaemia (SS).
3. *As double heterozygous states* e.g. sickle β -thalassaemia, sickle-C disease (SC), sickle-D disease (SD).

Heterozygous State: Sickle Cell Trait

Sickle cell trait is a benign heterozygous state of HbS in which only one abnormal gene is inherited. Patients with AS develop no significant clinical problems except when they become severely hypoxic and may develop sickle cell crises.

LABORATORY FINDINGS. These patients have no anaemia and have normal appearance of red cells. But in hypoxic crisis, sickle cell crises develop. The diagnosis is made by 2 tests:

1. *Demonstration of sickling* done under condition of reduced oxygen tension by an oxygen consuming reagent, sodium metabisulfite.
2. *Haemoglobin electrophoresis* reveals 35-40% of the total haemoglobin as HbS.

Homozygous State: Sickle Cell Anaemia

Sickle cell anaemia (SS) is a homozygous state of HbS in the red cells in which an abnormal gene is inherited from each parent. SS is a severely malignant disorder associated with protean clinical manifestations and decreased life expectancy.

PATHOGENESIS. Following abnormalities are observed (Fig. 33.18):

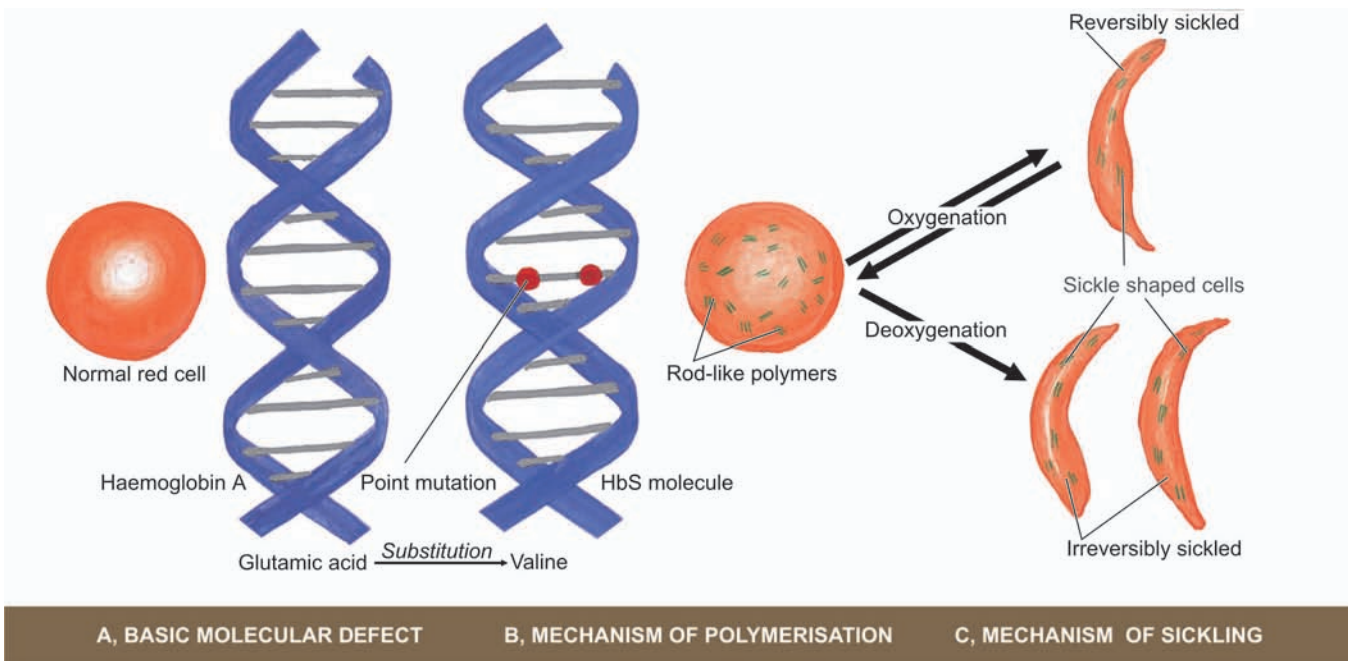


FIGURE 33.18

Pathogenesis of sickle cell anaemia. A, Basic molecular defect. B, Mechanism of polymerisation and consequent sickling of red cells containing HbS. C, Mechanism of sickling on oxygenation-deoxygenation.

1. Basic molecular lesion: In HbS, basic genetic defect is the *single point mutation* in one amino acid out of 146 in haemoglobin molecule; there is *substitution of valine for glutamic acid* at the 6 residue position of the β -globin, producing Hb $\alpha_2\beta_2^s$.

2. Mechanism of sickling: During deoxygenation, the red cells containing HbS change from biconcave disc shape to an elongated crescent-shaped or sickle-shaped cell. This process termed *sickling* occurs both within the intact red cells and *in vitro* in free solution. The mechanism responsible for sickling upon deoxygenation of HbS-containing red cells is the polymerisation of deoxygenated HbS which aggregates to form elongated rod-like polymers. These elongated fibres align and distort the red cell into classic sickle shape.

3. Reversible-irreversible sickling: The oxygen-dependent sickling process is usually reversible. However, damage to red cell membrane leads to formation of irreversibly sickled red cells even after they are exposed to normal oxygen tension.

4. Factors determining rate of sickling: The following factors determine the rate at which the polymerisation of HbS and consequent sickling take place:

i) *Presence of non-HbS haemoglobins:* The red cells in patients of SS have predominance of HbS and a small part consists of non-HbS haemoglobins, chiefly HbF

(2-20% of the total haemoglobin). HbF-containing red cells are protected from sickling while HbA-containing red cells participate readily in co-polymerisation with HbS.

ii) *Intracellular concentration of HbS.*

iii) *Total haemoglobin concentration.*

iv) *Extent of deoxygenation.*

v) *Acidosis and dehydration.*

vi) *Increased concentration of 2, 3-BPG in the red cells.*

CLINICAL FEATURES. The clinical manifestations of homozygous sickle cell disease are widespread. The symptoms begin to appear after 6th month of life when most of the HbF is replaced by HbS. Infection and folic acid deficiency result in more severe clinical manifestations. These features are as under:

1. Anaemia. There is usually severe chronic haemolytic anaemia (primarily extravascular) with onset of aplastic crisis in between. The symptoms of anaemia are generally mild since HbS gives up oxygen more readily than HbA to the tissues.

2. Vaso-occlusive phenomena. Patients of SS develop recurrent vaso-occlusive episodes throughout their lives due to obstruction to capillary blood flow by sickled red cells upon deoxygenation or dehydration. Vaso-

obstruction affecting different organs and tissues results in infarcts which may be of 2 types:

- i) *Microinfarcts* affecting particularly the abdomen, chest, back and joints and are the cause of recurrent painful crises in SS.
- ii) *Macroinfarcts* involving most commonly the spleen (splenic sequestration, autosplenectomy), bone marrow (pains), bones (aseptic necrosis, osteomyelitis), lungs (pulmonary infections), kidneys (renal cortical necrosis), CNS (stroke), retina (damage) and skin (ulcers), and result in anatomic and functional damage to these organs.

3. Constitutional symptoms. In addition to the features of anaemia and infarction, patients with SS have impaired growth and development and increased susceptibility to infection due to markedly impaired splenic function.

LABORATORY FINDINGS. The diagnosis of SS is considered almost exclusively in a Negro patient with haemolytic anaemia. The laboratory findings in these cases are:

1. Moderate to severe anaemia (haemoglobin concentration 6-9 g/dl).
2. The blood film shows sickle cells and target cells and features of splenic atrophy such as presence of Howell-Jolly bodies.
3. A positive sickling test with a reducing substance such as sodium metabisulfite (described above).
4. Haemoglobin electrophoresis shows no normal HbA but shows predominance of HbS and 2-20% HbF.

Double Heterozygous States

Double heterozygous conditions involving combination of HbS with other haemoglobinopathies may occur. Most common among these are sickle- β -thalassaemia, sickle C disease (SC), and sickle D disease (SD). All these disorders behave like mild form of sickle cell disease. Their diagnosis is made by haemoglobin electrophoresis and separating the different haemoglobins.

THALASSAEMIA

DEFINITION AND CLASSIFICATION

The thalassaemias are a diverse group of hereditary disorders in which there is reduced rate of synthesis of one or more of the globin polypeptide chains. Thus, thalassaemias, unlike haemoglobinopathies which are qualitative disorders of haemoglobin, are *quantitative abnormalities of polypeptide globin chain synthesis*.

Thalassaemias were first described in people of Mediterranean countries (North Africa, Southern Europe) from where it derives its name 'Mediterranean anaemia.' The Word '*thalassa*' in Greek means 'the sea' since the condition was found commonly in regions around the Mediterranean basin. It also occurs in the Middle East, India, South-East Asia and, in general in blacks.

Thalassaemias are genetically transmitted disorders. Normally, an individual inherits two β -globin genes located one each on two chromosomes 11, and two α -globin genes one each on two chromosomes 16, from each parent i.e. normal adult haemoglobin (HbA) is $\alpha_2\beta_2^*$. Depending upon whether the genetic defect or deletion lies in transmission of α - or β -globin chain genes, thalassaemias are classified into α - and β -thalassaemias. Thus, patients with α -thalassaemia have structurally normal α -globin chains but their production is impaired. Similarly, in β -thalassaemia, β -globin chains are structurally normal but their production is decreased. Each of the two main types of thalassaemias may occur as heterozygous (called α - and β -thalassaemia *minor* or *trait*), or as homogygous state (termed α - and β -thalassaemia *major*). The former is generally asymptomatic, while the latter is a severe congenital haemolytic anaemia.

A classification of various types of thalassaemias alongwith the clinical syndromes produced and salient laboratory findings are given in Table 33.13.

PATHOPHYSIOLOGY OF ANAEMIA IN THALASSAEMIA

A constant feature of all forms of thalassaemia is the presence of anaemia.

α -Thalassaemia: In α -thalassaemia major, the obvious cause of anaemia is the inability to synthesise adult haemoglobin, while in α -thalassaemia trait there is reduced production of normal adult haemoglobin.

β -Thalassaemia: In β -thalassaemia major, the most important cause of anaemia is premature red cell destruction brought about by erythrocyte membrane damage caused by the precipitated α -globin chains. Other contributory factors are: shortened red cell life-span, ineffective erythropoiesis, and haemodilution due to increased plasma volume. A deficiency of β -globin chains in β -thalassaemia leads to large excess of α -chains within

*In a normal adult, distribution of haemoglobin is as under: HbA ($\alpha_2\beta_2$) = 95-98%, HbA₂ ($\alpha_2\delta_2$) (a minor variant of HbA) = 1.5-3.5%, HbF ($\alpha_2\gamma_2$) = less than 1%. But the level of HbF in children under 6 months is slightly higher.

TABLE 33.13: Classification of Thalassaemias.

TYPE	HB	HB-ELECTROPHORESIS	GENOTYPE	CLINICAL SYNDROME
α-Thalassaemias				
1. <i>Hydrops foetalis</i>	3-10 g/dl	Hb Barts (γ_4) (100%)	Deletion of four α -genes	Fatal <i>in utero</i> or in early infancy
2. <i>Hb-H disease</i>	2-12 g/dl	HbF (10%)	Deletion of three α -genes	Haemolytic anaemia
3. <i>α-Thalassaemia trait</i>	10-14 g/dl	Normal	Deletion of two α -genes	Microcytic hypochromic blood picture but no anaemia
β-Thalassaemias				
1. <i>β-Thalassaemia major</i>	< 5 g/dl	HbA (0-50%), HbF(50-98%)	$\beta^{\text{thal}}/\beta^{\text{thal}}$	Severe congenital haemolytic anaemia, requires blood transfusions
2. <i>β-Thalassaemia intermedia</i>	5-10 g/dl	Variable	Multiple mechanisms	Severe anaemia, but regular blood transfusions not required
3. <i>β-Thalassaemia minor</i>	10-12 g/dl	HbA ₂ (4-9%) HbF (1-5%)	$\beta^{\text{A}}/\beta^{\text{thal}}$	Usually asymptomatic

the developing red cells. Part of these excessive α -chains are removed by pairing with γ -globin chains as HbF, while the remainder unaccompanied α -chains precipitate rapidly within the red cell as *Heinz bodies*. The precipitated α -chains cause cell membrane damage. During their passage through the splenic sinusoids, these red cells are further damaged and develop pitting due to removal of the precipitated aggregates. Thus, such red cells are irreparably damaged and are phagocytosed by the RE cells of the spleen and liver causing anaemia, hepatosplenomegaly, and excess of tissue iron stores. Patients with β -thalassaemia minor, on the other hand, have very mild ineffective erythropoiesis, haemolysis and shortening of red cell life-span.

α -THALASSAEMIA

MOLECULAR PATHOGENESIS. α -thalassaemias are disorders in which there is defective synthesis of α -globin chains resulting in depressed production of haemoglobins that contain α -chains i.e. HbA, HbA₂ and HbF. The α -thalassaemias are most commonly due to deletion of one or more of the α -chain genes located on short arm of chromosome 16. Since there is a pair of α -chain genes, the clinical manifestations of α -thalassaemia depend upon the number of genes deleted. Accordingly, α -thalassaemias are classified into 4 types:

1. Four α -gene deletion: Hb Bart's hydrops foetalis.
2. Three α -gene deletion: HbH disease.
3. Two α -gene deletion: α -thalassaemia trait.
4. One α -gene deletion: α -thalassaemia trait (carrier).

Hb Bart's Hydrops Foetalis

When there is deletion of all the four α -chain genes (homozygous state) it results in total suppression of α -globin chain synthesis causing the most severe form of α -thalassaemia called Hb Bart's hydrops foetalis. Hb Bart's is a gamma globin chain tetramer (γ_4) which has high oxygen affinity leading to severe tissue hypoxia.

CLINICAL FEATURES. Hb Bart's hydrops foetalis is incompatible with life due to severe tissue hypoxia. The condition is either fatal *in utero* or the infant dies shortly after birth. If born alive, the features of severe Rh haemolytic disease are present (page 516).

LABORATORY FINDINGS. Infants with Hb Bart's hydrops foetalis born alive may have the following laboratory findings:

1. Severe anaemia (haemoglobin below 6g/dl).
2. Blood film show marked anisopoikilocytosis, hypochromia, microcytosis, polychromasia, basophilic stippling, numerous normoblasts and target cells.
3. Reticulocyte count is high.
4. Serum bilirubin level is elevated.
5. Haemoglobin electrophoresis shows 80-90% Hb-Bart's and a small amount of Hb-H and Hb-Portland but no HbA, HbA₂ or HbF.

HbH Disease

Deletion of three α -chain genes produces HbH which is a β -globin chain tetramer (β_4) and markedly impaired α -chain synthesis. HbH is precipitated as Heinz bodies

within the affected red cells. An elongated α -chain variant of HbH disease is termed *Hb Constant Spring*.

CLINICAL FEATURES. HbH disease is generally present as a well-compensated haemolytic anaemia. The features are intermediate between that of β -thalassaemia minor and major. The severity of anaemia fluctuates and may fall to very low levels during pregnancy or infections. Majority of patients have splenomegaly and may develop cholelithiasis.

LABORATORY FINDINGS. These are:

1. Moderate anaemia (haemoglobin 8-9 g/dl).
2. Blood film shows severe microcytosis, hypochromia, basophilic stippling, target cells and normoblasts.
3. Mild reticulocytosis.
4. HbH inclusions as Heinz bodies can be demonstrated in mature red cells with brilliant cresyl blue stain.
5. Haemoglobin electrophoresis shows 2-4% HbH and the remainder consists of HbA, HbA₂ and HbF.

α -Thalassaemia Trait

The α -thalassaemia trait may occur by the following molecular pathogenesis:

- By deletion of two of the four α -chain genes in homozygous form called *homozygous α -thalassaemia*, or in double heterozygous form termed *heterozygous α -thalassaemia*.
- By deletion of a single α -chain gene causing heterozygous α -thalassaemia trait called *heterozygous α -thalassaemia*.

CLINICAL FEATURES. The α -thalassaemia trait due to two α -chain gene deletion is asymptomatic. It is suspected in a patient of refractory microcytic hypochromic anaemia in whom iron deficiency and β -thalassaemia minor have been excluded and the patient belongs to the high-risk ethnic group. One gene deletion α -thalassaemia trait is a silent carrier state.

LABORATORY FINDINGS. The patients of α -thalassaemia trait may have the following haematological findings:

1. Haemoglobin level normal or mildly reduced.
2. Blood film shows microcytic and hypochromic red cell morphology but no evidence of haemolysis or anaemia.
3. MCV, MCH and MCHC may be slightly reduced.
4. Haemoglobin electrophoresis reveals small amount of Hb-Bart's in neonatal period (1-2% in α -thalassaemia 2 and 5-6% in α -thalassaemia 1) which

gradually disappears by adult life. HbA₂ is either normal or slightly decreased (contrary to the elevated HbA₂ levels in β -thalassaemia trait).

β -THALASSAEMIAS

MOLECULAR PATHOGENESIS. The β -thalassaemias are caused by decreased rate of β -chain synthesis resulting in reduced formation of HbA in the red cells. The molecular pathogenesis of the β -thalassaemias is more complex than that of α -thalassaemias. In contrast to α -thalassaemia, gene deletions rarely ever cause β -thalassaemia and is only seen in an entity called *hereditary persistence of foetal haemoglobin (HPFH)*. Instead, most of β -thalassaemias arise from different types of mutations of β -globin gene resulting from single base changes. The symbol β^0 is used to indicate the complete absence of synthesis while β^+ denotes partial synthesis of the β -globin chains. More than 100 such mutations have been described affecting the preferred sites in the coding sequences e.g. in promoter region, termination region, splice junctions, exons, introns). Some of the important ones having effects on β -globin chain synthesis are as under:

- i) *Transcription defect:* Mutation affecting transcriptional promoter sequence causing reduced synthesis of β -globin chain. Hence the result is partially preserved synthesis i.e. β^+ *thalassaemia*.
- ii) *Translation defect:* Mutation in the coding sequence causing stop codon (chain termination) interrupting β -globin messenger RNA. This would result in no synthesis of β -globin chain and hence β^0 *thalassaemia*.
- iii) *mRNA splicing defect:* Mutation leads to defective mRNA processing forming abnormal mRNA that is degraded in the nucleus. Depending upon whether part of splice site remains intact or is totally degraded, it may result in β^+ *thalassaemia* or β^0 *thalassaemia*.

Depending upon the extent of reduction in β -chain synthesis, there are 3 types of β -thalassaemia:

1. Homozygous form: β -Thalassaemia major. It is the most severe form of congenital haemolytic anaemia. It is further of 2 types (Fig. 33.19):

- i) β^0 *thalassaemia major* characterised by complete absence of β -chain synthesis.
- ii) β^+ *thalassaemia major* having incomplete suppression of β -chain synthesis.

2. β -Thalassaemia intermedia: It is β -thalassaemia of intermediate degree of severity that does not require regular blood transfusions. These cases are genetically heterozygous (β^0/β or β^+/β).

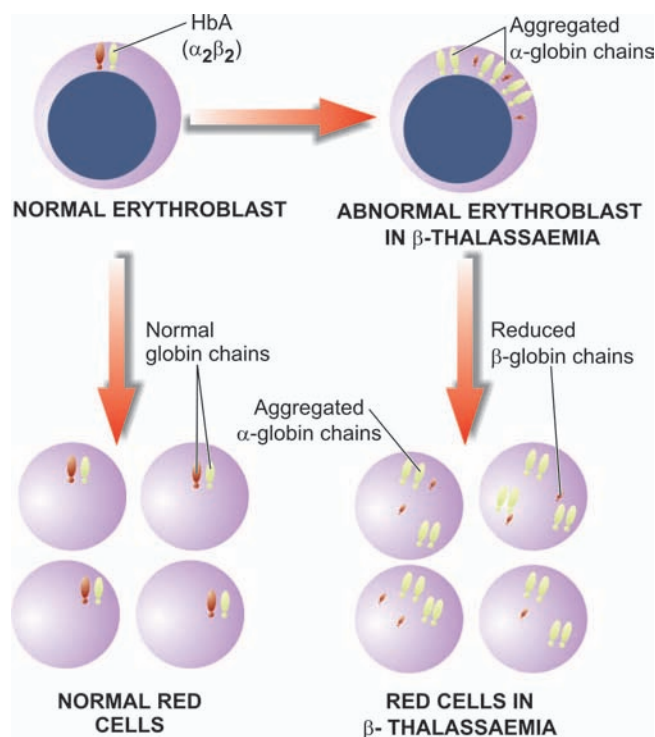


FIGURE 33.19

Pathogenesis of β -thalassaemia major.

3. Heterozygous form: β -Thalassaemia minor (trait). It is a mild asymptomatic condition in which there is moderate suppression of β -chain synthesis.

Besides β -thalassaemia minor, a few uncommon globin chain combinations resulting in β -thalassaemia trait are as under:

- i) $\delta\beta$ -thalassaemia minor in which there is total absence of both β and δ chain synthesis and is characterised by elevated HbF level but unlike β -thalassaemia minor there is normal or reduced HbA₂ level.
- ii) *Hb Lepore syndrome* characterised by nonhomologous fusion of β - and δ -genes forming an abnormal haemoglobin called Hb Lepore (named after a family called Lepore). There is total absence of normal β -chain synthesis.

An individual may inherit one β -chain gene from each parent and produce heterozygous, homozygous, or double heterozygous states. Statistically, 25% of offsprings born to two heterozygotes (i.e. β -thalassaemia trait) will have the homozygous state i.e. β -thalassaemia major.

β -Thalassaemia Major

The β -thalassaemia major, also termed Mediterranean or Cooley's anaemia is the most common form of conge-

nital haemolytic anaemia. More commonly, β -thalassaemia major is a homozygous state with either complete absence of β -chain synthesis (β^0 *thalassaemia major*) or only small amounts of β -chains are formed (β^+ *thalassaemia major*). These result in excessive formation of alternate haemoglobins, HbF ($\alpha_2\gamma_2$) and HbA₂ ($\alpha_2\delta_2$).

CLINICAL FEATURES. Clinical manifestations appear insidiously and are as under (Fig. 33.20):

1. Anaemia starts appearing within the first 4-6 months of life when the switch over from γ -chain to β -chain production occurs.
2. Marked hepatosplenomegaly occurs due to excessive red cell destruction, extramedullary haematopoiesis and iron overload.
3. Expansion of bones occurs due to marked erythroid hyperplasia leading to thalassaemic facies and malocclusion of the jaw.
4. Iron overload due to repeated blood transfusions causes damage to the endocrine organs resulting in slow rate of growth and development, delayed puberty, diabetes mellitus and damage to the liver and heart.

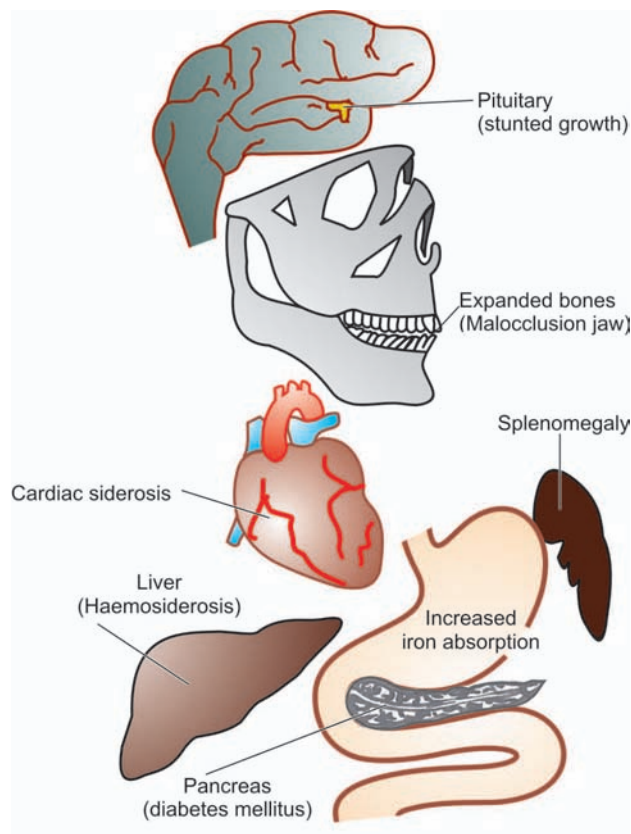


FIGURE 33.20

Major clinical features in β -thalassaemia major.

LABORATORY FINDINGS. The haematological investigations reveal the following findings (**COLOUR PLATE X: CL 39**):

1. *Anaemia*, usually severe.
2. *Blood film* shows severe microcytic hypochromic red cell morphology, marked anisopoikilocytosis, basophilic stippling, presence of many target cells, tear drop cells and normoblasts (Fig. 33.21,A).
3. *Serum bilirubin* (unconjugated) is generally raised.
4. *Reticulocytosis* is generally present.
5. *MCV, MCH and MCHC* are significantly reduced.
6. *WBC count* is often raised with some shift to left of the neutrophil series, with presence of some myelocytes and metamyelocytes.
7. *Platelet count* is usually normal but may be reduced in patients with massive splenomegaly.
8. Osmotic fragility characteristically reveals increased resistance to saline haemolysis i.e. *decreased osmotic fragility* (Fig. 33.21,B).
9. *Haemoglobin electrophoresis* shows presence of increased amounts of HbF, increased amount of HbA₂, and almost complete absence or presence of variable amounts of HbA. The increased level of HbA₂ has not been found in any other haemoglobin abnormality except β -thalassaemia. The increased synthesis of HbA₂ is probably due to increased activity at both δ -chain loci.

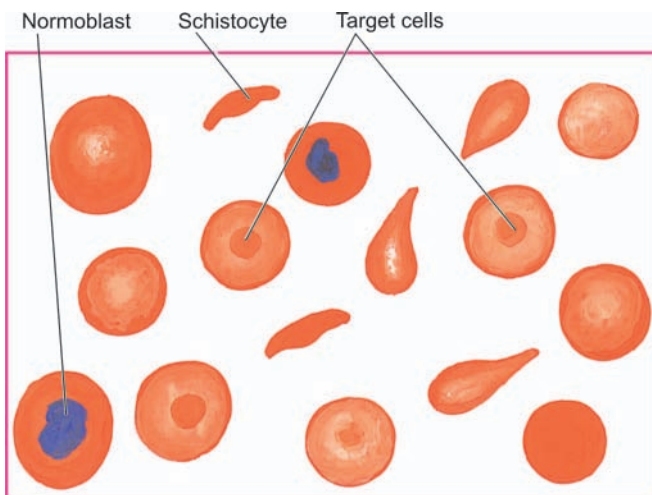
10. *Bone marrow aspirate examination* shows normoblastic erythroid hyperplasia with predominance of intermediate and late normoblasts which are generally smaller in size than normal. Iron staining demonstrates siderotic granules in the cytoplasm of normoblasts, increased reticuloendothelial iron but ring sideroblasts are only occasionally seen.

Treatment

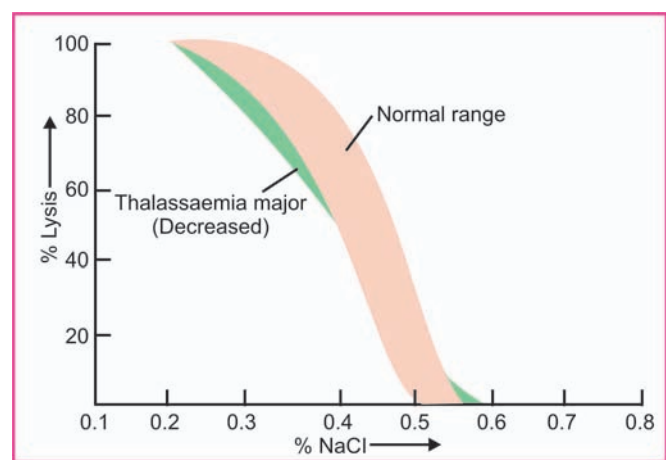
Treatment of β -thalassaemia major is largely supportive.

1. Anaemia is generally severe and patients require regular blood transfusions (4-6 weekly) to maintain haemoglobin above 8 g/dl.
2. In order to maintain increased demand of hyperplastic marrow, folic acid supplement is given.
3. Splenectomy is beneficial in children over 6 years of age since splenic sequestration contributes to shortened red cell life-span.
4. Prevention and treatment of iron overload is done by chelation therapy (desferrioxamine). Oral chelation with kelfer or deferiprone is also available now.
5. Bone marrow transplantation from HLA-matched donor is being done in many centres with fair success rate.
6. Some workers have found success with cord blood transfusion.

Since these patients require multiple blood transfusions, they are at increased risk of developing AIDS.



A, BLOOD FILM IN β -THALASSAEMIA MAJOR



B, OSMOTIC FRAGILITY CURVE

FIGURE 33.21

Laboratory findings in β -thalassaemia major. A, Findings in peripheral blood film. B, Osmotic fragility test showing decreased fragility.

In general, patients with β -thalassaemia major have short life expectancy and it is unusual for a patient with severe form of the disease to survive into adulthood. The biggest problem is iron overload and consequent myocardial siderosis leading to cardiac arrhythmias, congestive heart failure, and ultimately death.

β -Thalassaemia Minor

The β -thalassaemia minor or β -thalassaemia trait, a heterozygous state, is a common entity characterised by moderate reduction in β -chain synthesis.

CLINICAL FEATURES. Clinically, the condition is usually asymptomatic and the diagnosis is generally made when the patient is being investigated for a mild chronic anaemia. The spleen may be palpable.

LABORATORY FINDINGS. These are as under:

1. *Mild anaemia*; mean haemoglobin level is about 15% lower than in normal person for the age and sex.
2. *Blood film* shows mild anisopoikilocytosis, microcytosis and hypochromia, occasional target cells and basophilic stippling.
3. *Serum bilirubin* may be normal or slightly raised.
4. *Mild reticulocytosis* is often present.
5. *MCV, MCH and MCHC* may be slightly reduced.
6. *Osmotic fragility test* shows increased resistance to haemolysis i.e. *decreased osmotic fragility*.
7. *Haemoglobin electrophoresis* is confirmatory for the diagnosis and shows about two-fold increase in HbA_2 and a slight elevation in HbF (2-3%).

Treatment

Patients with β -thalassaemia minor do not require any treatment. But they should be explained about the genetic implications of the disorder, particularly to those of child-bearing age. If the two subjects of β -thalassaemia trait marry, there is a 25% chance of developing thalassaemia major in offsprings. Patients with β -thalassaemia minor have normal life expectancy but those who are treated under the mistaken diagnosis of iron deficiency may develop siderosis and its complications.

Prevention of Thalassaemia

Finally, since thalassaemia is an inheritable disease, its prevention is possible by making an antenatal diagnosis. This is done by chorionic villous biopsy or cells obtained by amniocentesis and DNA studied by PCR amplification technique for presence of genetic mutations of thalassaemias.

APLASTIC ANAEMIA AND OTHER PRIMARY BONE MARROW DISORDERS

'Bone marrow failure' is the term used for primary disorders of the bone marrow which result in impaired formation of the erythropoietic precursors and consequent anaemia. It includes the following disorders:

1. *Aplastic anaemia*, most importantly.
2. *Other primary bone marrow disorders* such as: myelophthisic anaemia, pure red cell aplasia, and myelodysplastic syndromes.

APLASTIC ANAEMIA

Aplastic anaemia is defined as pancytopenia (i.e. simultaneous presence of anaemia, leucopenia and thrombocytopenia) resulting from aplasia of the bone marrow. The underlying defect in all cases appears to be sufficient reduction in the number of haematopoietic pluripotent stem cells which makes them unable to divide and differentiate.

ETIOLOGY AND CLASSIFICATION. Based on the etiology, aplastic anaemia is classified into 2 main types: primary and secondary. The various causes that may give rise to both these types of aplastic anaemia are summarised in Table 33.14 and briefly considered below:

A. Primary aplastic anaemia. Primary type of aplastic anaemia includes 2 entities: a congenital form called Fanconi's anaemia and an immunologically-mediated acquired form.

1. *Fanconi's anaemia*. This has an autosomal recessive inheritance and is often associated with other congenital anomalies such as skeletal and renal abnormalities, and sometimes mental retardation.

TABLE 33.14: Causes of Aplastic Anaemia.

A. PRIMARY APLASTIC ANAEMIA

1. *Fanconi's anaemia (congenital)*
2. *Immunologically-mediated (acquired)*

B. SECONDARY APLASTIC ANAEMIA

1. *Drugs*
 - i) Dose-related aplasia e.g. with antimetabolites (methotrexate), mitotic inhibitors (daunorubicin), alkylating agents (busulfan), nitroso urea, anthracyclines.
 - ii) Idiosyncratic aplasia e.g. with chloramphenicol, sulfa drugs, oxyphenbutazone, phenylbutazone, chlorpromazine, gold salts.
2. *Toxic chemicals* e.g. benzene derivatives, insecticides, arsenicals.
3. *Infections* e.g. infectious hepatitis, EB virus infection, AIDS, other viral illnesses.
4. *Miscellaneous* e.g. association with SLE and therapeutic X-rays

2. *Immune causes.* In many cases, suppression of haematopoietic stem cells by immunologic mechanisms may cause aplastic anaemia. The observations in support of autoimmune mechanisms are the clinical response to immunosuppressive therapy and *in vitro* marrow culture experiments.

B. Secondary aplastic anaemia. Aplastic anaemia may occur secondary to a variety of industrial, physical, chemical, iatrogenic and infectious causes.

1. *Drugs.* A number of drugs are cytotoxic to the marrow and cause aplastic anaemia. The association of a drug with aplastic anaemia may be either predictably dose-related or an idiosyncratic reaction.

■ *Dose-related aplasia* of the bone marrow occurs with antimetabolites (e.g. methotrexate), mitotic inhibitors (e.g. daunorubicin), alkylating agents (e.g. busulfan), nitroso urea and anthracyclines. In such cases, withdrawal of the drug usually allows recovery of the marrow elements.

■ *Idiosyncratic aplasia* is depression of the bone marrow due to qualitatively abnormal reaction of an individual to a drug when first administered. The most serious and most common example of idiosyncratic aplasia is associated with chloramphenicol. Other such common drugs are: sulfa drugs, oxyphenbutazone, phenylbutazone, chlorpromazine, gold salts etc.

2. *Toxic chemicals.* These include examples of industrial, domestic and accidental use of substances such as benzene derivatives, insecticides, arsenicals etc.

3. *Infections.* Aplastic anaemia may occur following viral hepatitis, Epstein-Barr virus infection, AIDS and other viral illnesses.

4. *Miscellaneous.* Lastly, aplastic anaemia has been reported in association with certain other illnesses such as SLE, and with therapeutic X-rays.

CLINICAL FEATURES. The onset of aplastic anaemia may occur at any age and is usually insidious. The clinical manifestations include the following:

1. Anaemia and its symptoms like mild progressive weakness and fatigue.
2. Haemorrhage from various sites due to thrombocytopenia such as from the skin, nose, gums, vagina, bowel, and occasionally in the CNS and retina.
3. Infections of the mouth and throat are commonly present.
4. The lymph nodes, liver and spleen are generally not enlarged.

LABORATORY FINDINGS. The diagnosis of aplastic anaemia is made by a thorough laboratory

evaluation and excluding other causes of pancytopenia (Table 33.15). The following haematological features are found:

1. Anaemia. Haemoglobin levels are moderately reduced. The blood picture generally shows normocytic normochromic anaemia but sometimes macrocytosis may be present. The reticulocyte count is reduced or zero.

2. Leucopenia. The absolute granulocyte count is particularly low (below 1500/ μ l) with relative lymphocytosis. The neutrophils are morphologically normal but their alkaline phosphatase score is high.

3. Thrombocytopenia. Platelet count is always reduced.

4. Bone marrow aspiration. A bone marrow aspirate may yield a 'dry tap'. A trephine biopsy is generally essential for making the diagnosis which reveals patchy cellular areas in a hypocellular or aplastic marrow due to replacement by fat. There is usually a severe depression of myeloid cells, megakaryocytes and erythroid cells so that the marrow chiefly consists of lymphocytes and plasma cells.

Treatment

The patients of mild aplasia may show spontaneous recovery, while the management of severe aplastic anaemia is a most challenging task. In general, younger patients show better response to proper treatment. The broad outlines of the treatment are as under:

A. General management: It consists of the following:

1. Identification and elimination of the possible cause.
2. Supportive care consisting of blood transfusions, platelet concentrates, and treatment and prevention of infections.

TABLE 33.15: Causes of Pancytopenia.

I. Aplastic anaemia (Table 33.14)
II. Pancytopenia with normal or increased marrow cellularity e.g.
1. Myelodysplastic syndromes
2. Hypersplenism
3. Megaloblastic anaemia
III. Paroxysmal nocturnal haemoglobinuria (page 467)
IV. Bone marrow infiltrations e.g.
1. Haematologic malignancies (leukaemias, lymphomas, myeloma)
2. Non-haematologic metastatic malignancies
3. Storage diseases
4. Osteopetrosis
5. Myelofibrosis

TABLE 33.16: French-American-British (FAB) Classification of Myelodysplastic Syndromes (MDS).

FINDINGS	RA	RARS	RAEB	CMML	RAEB-t
A. Incidence	28%	24%	23%	16%	9%
B. Peripheral Blood					
1. Haemoglobin	Low	Low	Low	Variable	Low
2. Myeloblasts	0-1%	0-1%	<5%	<5%	>5%
3. Monocytes	Rare	Rare	Rare	>1000/ μ l	Variable
C. Bone marrow					
1. Myeloblasts	<5%	<5%	5-20%	1-20%	20-30%
2. Ringed sideroblasts	$\pm$	>15%	$\pm$	$\pm$	$\pm$

Abbreviations: RA= refractory anaemia; RARS = refractory anaemia with ringed sideroblasts; RAEB = refractory anaemia with excess blasts; CMML= chronic myelomonocytic leukaemia; RAEB-t= refractory anaemia with excess blasts in transformation.

B. Specific treatment: The specific treatment has been attempted with varying success and includes the following:

1. *Marrow stimulating agents* such as androgen may be administered orally.
2. *Immunosuppressive therapy* with agents such as anti-thymocyte globulin and anti-lymphocyte serum has been tried with 40-50% success rate. Very high doses of glucocorticoids or cyclosporine may yield similar responses. But splenectomy does not have any role in the management of aplastic anaemia.
3. *Bone marrow transplantation* is considered in severe cases under the age of 40 years where the HLA and mixed culture-matched donor is available. (*Other indications* for bone marrow transplantation are: acute leukaemias—AML in first remission and ALL in second remission, thalassaemia major and combined immuno-deficiency disease). Complications of bone marrow transplantation such as severe infections, graft rejection and graft-versus-host disease (GVHD) may occur.

Severe aplastic anaemia is a serious disorder terminating in death within 6-12 months in 50-80% of cases. Death is usually due to bleeding and/or infection.

MYELOYDYSPLASTIC SYNDROMES

Myelodysplastic syndromes (MDS) or *preleukaemic syndromes* are a heterogeneous group of leukaemia-related conditions having abnormalities in development of different marrow elements in varying combinations. These conditions are, therefore, also termed as *dysmyelopoietic syndromes*. The various combinations of features include normocytic normochromic anaemia, presence of certain number of myeloblasts in the peripheral blood, neutropenia, thrombocytopenia and/or monocytosis. The bone marrow reveals disordered maturation of lymphoid, myeloid and megakaryocytic cells.

The FAB (French-American-British) classification

divides myelodysplastic syndromes into the following 5 groups (Table 33.16):

1. Refractory anaemia (RA).
2. Refractory anaemia with ringed sideroblasts (primary acquired sideroblastic anaemia) (RARS).
3. Refractory anaemia with excess blasts (RAEB).
4. Chronic myelomonocytic leukaemia (CMML).
5. Refractory anaemia with excess of blasts in transformation (RAEB-t).

As per FAB classification, marrow may contain up to 30% myeloblasts in MDS and was considered as the dividing line for distinguishing cases of AML from MDS. However, as per recently described *WHO classification*, patients with blast count of 20-30% and labelled as RAEB-t (group 5 above) in FAB classification have prognosis similar to patients with blast count above 30% (i.e. AML cases). Thus, as per WHO classification, marrow blast count for making the diagnosis of AML has been revised and brought down to 20%. Patients with FAB category of RAEB-t are currently considered and treated as cases of AML and the term RAEB-t stands excluded from MDS according to the WHO classification.

MDS is found more frequently in older people (above 50 years of age). The etiology remains unknown but an association with chemotherapy or radiation has been observed in some cases. Many of the features are like those of acute leukaemia but the clinical course in myelodysplastic syndromes tends to be more chronic. However, 5-20% patients actually evolve into frank acute myeloid leukaemia (with more than 30% blasts in the bone marrow), confirming their preleukaemic nature.

Survival in different subtypes ranges from 3-76 months, patients either succumbing to infections or developing into acute myeloid leukaemia. Patients with RA and RARS have a better prognosis while patients with RAEB and RAEB-t have a higher rate of leukaemic conversion and a shorter survival.



The leucocytes of the peripheral blood are of 2 main varieties, distinguished by the presence or absence of granules. These are: *granulocytes* and *nongranular leucocytes*. The granulocytes, according to the appearance of nuclei, are subdivided into polymorphonuclear leucocytes and monocytes. Depending upon the colour of granules, polymorphonuclear leucocytes are further of 3 types: neutrophils, eosinophils and basophils. The nongranular leucocytes are lymphocytes.

GRANULOPOIESIS

Site of Formation and Kinetics

All forms of granulocytes are produced in the bone marrow and are termed, '*myeloid series*'. These are: myeloblast (most primitive precursor), promyelocyte, myelocyte, metamyelocyte, band forms and segmented granulocyte (mature form). The myeloblast, promyelocyte and myelocyte form a '*proliferative or mitotic pool*', while the latter series i.e. metamyelocyte, band forms and segmented granulocytes make up a '*mature or post-mitotic pool*'. It takes about 12 days for formation of mature granulocytes from the myeloblast. Normally the bone marrow contains more myeloid cells than the erythroid cells in the ratio of 2:1 to 15:1 (average 3:1), the largest proportion being that of metamyelocytes, band forms and segmented neutrophils.

Normally, the bone marrow storage compartment contains about 10-15 times the number of granulocytes found in the peripheral blood. Following their release from the bone marrow, granulocytes spend about 10 hours in the circulation before they move into the tissues, where they perform their function of phagocytosis. The blood pool of granulocytes consists of 2 components of about equal size—the *circulating pool* that is included in the blood count, and the *marginating pool* that is not included in the blood count. Granulocytes spend about 4-5 days in the tissues before they are either destroyed during phagocytosis or die due to senescence (Fig. 34.1). To control the various compartments of granulocytes, a 'feed-back system' exists between the circulating and tissue granulocytes on one side, and the marrow granulocytes on the other. The presence of a humoral regulatory substance, 'granulopoietin' analogous to

erythropoietin has also been identified by *in vitro* studies of colony-forming units (CFU) and is characterised as G-CSF (granulocyte colony-stimulating factor) and GM-CSF (granulocyte-monocyte colony-stimulating factor).

The kinetics of monocytes is less well understood than that of other myeloid cells. Monocytes spend about 20-40 hours in the circulation after which they leave the blood to enter extravascular tissues where they perform their main function of active phagocytosis. The extravascular life-span of tissue macrophages which are the transformed form of blood monocytes, may vary from a few months to a few years.

Myeloid Series

The development of myeloid cells from myeloblast takes place in the following sequence (Fig. 34.2):

1. MYELOBLAST. The myeloblast is the earliest recognisable precursor of the granulocytes, normally comprising about 2% of the total marrow cells. The myeloblast varies considerably in size (10-18 μm in diameter), having a large round to oval nucleus nearly filling the cell, has fine nuclear chromatin and contains 2-5 well-defined pale nucleoli. The thin rim of cytoplasm is deeply basophilic and devoid of granules. The myeloblasts of acute myeloid leukaemia may, however, show the presence of rod-like cytoplasmic inclusions called *Auer's rods* which represent abnormal derivatives of primary azurophilic granules.

The nuclei of successive stages during their development from myeloblast become progressively coarser and lose their nucleoli and the cytoplasm loses its blue colour. As the cells become mature the granules appear; firstly non-specific azurophilic granules appear which are followed by specific granules that differentiate the neutrophils, eosinophils and basophils.

2. PROMYELOCYTE. The promyelocyte is slightly larger than the myeloblast (12-18 μm diameter). It possesses a round to oval nucleus, having fine nuclear chromatin which is slightly condensed around the nuclear membrane. The nucleoli are present but are less prominent and fewer than those in the myeloblast. The main distinction of promyelocyte from myeloblast is in the cytoplasm which contains azurophilic (primary or non-specific) granules.

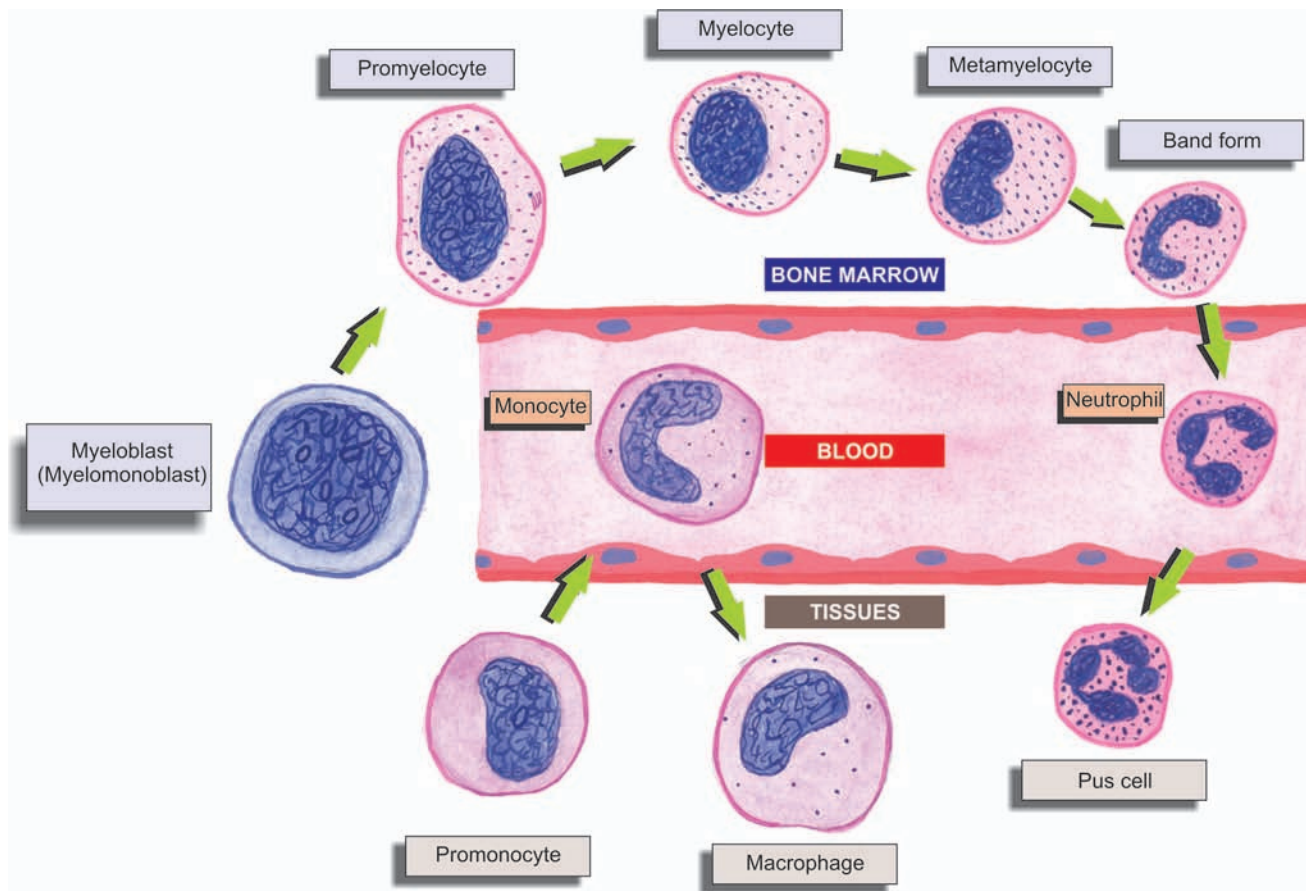


FIGURE 34.1

Granulopoiesis and the cellular compartments of myeloid cells in the bone marrow, blood and tissues.

3. MYELOCYTE. The myelocyte is the stage in which specific or secondary granules appear in the cytoplasm, and accordingly, the cell can be identified at this stage as belonging to the neutrophilic, eosinophilic or basophilic myelocyte. Primary granules also persist at this stage but formation of new primary granules stops. The nucleus of myelocyte is eccentric, round to oval, having

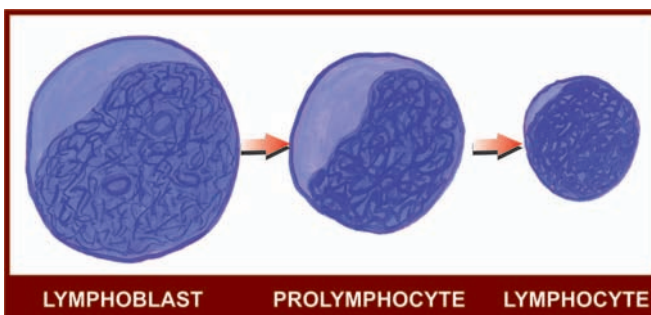


FIGURE 34.2

The formation of lymphoid series of cells.

somewhat coarse nuclear chromatin and no visible nucleoli. The myeloid cells up to the myelocyte stage continue to divide and, therefore, comprise *mitotic or proliferative pool*.

4. METAMYELOCYTE. The metamyelocyte stage is 10-18 μm in diameter and is characterised by a clearly indented or horseshoe-shaped nucleus without nucleoli. The nuclear chromatin is dense and clumped. The cytoplasm contains both primary and secondary granules. The metamyelocytes are best distinguished from the monocytes by the clumped nuclear chromatin while the latter have fine chromatin.

5. BAND FORMS. Band form is juvenile granulocyte, 10-16 μm in diameter, characterised by further condensation of nuclear chromatin and transformation of nuclear shape into band configuration of uniform thickness.

6. SEGMENTED GRANULOCYTES. The mature polymorphonuclear leucocytes, namely: the neutrophils, eosinophils and basophils, are described later on page 484.

Monocyte-Macrophage Series

The monocyte-macrophage series of cells, though comprise a part of myeloid series along with other granulocytic series, but are described separately here in view of different morphologic stages in their maturation (Fig. 34.1).

- 1. MONOBLAST.** The monoblast is the least mature of the recognisable cell of monocyte-macrophage series. It is very similar in appearance to myeloblasts except that it has ground-glass cytoplasm with irregular border and may show phagocytosis as indicated by the presence of engulfed red cells in the cytoplasm. However, differentiation from myeloblast at times may be difficult even by electron microscopy and, therefore, it is preferable to call the earliest precursor of granulocytic series as *myelomonoblast*.
- 2. PROMONOCYTE.** The promonocyte is a young monocyte, about 20 μm in diameter and possesses a large indented nucleus containing a nucleolus. The cytoplasm is basophilic and contains no azurophilic granules but may have fine granules which are larger than those in the mature monocyte.
- 3. MONOCYTE.** The mature form of monocytic series is described on page 486, while the transformed stages of these cells in various tissues (i.e. macrophages) are a part of RE system discussed on page 109.

LYMPHOPOIESIS

Sites of Formation and Kinetics

The lymphocytes and the plasma cells are immunocompetent cells of the body. In man, the bone marrow and the thymus are the *primary lymphopoietic organs* where lymphoid stem cells undergo spontaneous division independent of antigenic stimulation. The *secondary or reactive lymphoid tissue* is comprised by the lymph nodes, spleen and gut-associated lymphoid tissue (GALT). These sites actively produce lymphocytes from the germinal centres of lymphoid follicles as a response to antigenic stimulation. Lymphocytes pass through a series of developmental changes in the course of their evolution into lymphocyte subpopulations and subsets. It includes migration of immature lymphocytes to other organs such as the thymus where locally-produced factors act on them.

Functionally, the lymphocytes are divided into T and B lymphocytes depending upon whether they are immunologically active in cell-mediated immunity or in humoral antibody response respectively. In man, the B cells are derived from the bone marrow stem cells,

while in birds they mature in the bursa of Fabricius. After antigenic activation, the B cells proliferate and mature into plasma cells which secrete specific immunoglobulin antibodies. The T cells are also produced in the bone marrow and possibly in the thymus. The concept of T and B lymphocytes along with their subpopulations is discussed on page 146.

Lymphoid Series

The maturation stages in production of lymphocytes are illustrated in Fig. 34.2 and are as under:

- 1. LYMPHOBLAST.** The lymphoblast is the earliest identifiable precursor of lymphoid cells and is a rapidly dividing cell. It is a large cell, 10-18 μm in diameter, containing a large round to oval nucleus having slightly clumped or stippled nuclear chromatin. The nuclear membrane is denser and the number of nucleoli is fewer (1-2) as compared with those in myeloblast (2-5). The cytoplasm is scanty, basophilic and non-granular.
- The distinguishing morphologic features between the myeloblast and lymphoblast are summarised in Table 34.1.
- 2. PROLYMPHOCYTE.** This stage is an intermediate stage between the lymphoblast and mature lymphocyte. These young lymphocytes are 9-18 μm in diameter, contain round to indented nucleus with slightly stippled or coarse chromatin and may have 0-1 nucleoli.
- 3. LYMPHOCYTE.** The mature lymphocytes are described below.

MATURE LEUCOCYTES IN HEALTH AND DISEASE

Normally, only mature leucocytes namely: polymorphs, lymphocytes, monocytes, eosinophils and basophils, are found in the peripheral blood. The normal range of total and differential leucocyte count (TLC and DLC) in health in adults and children is given in Table 34.2. White cell count tends to be higher in infants and children than in adults. It also normally undergoes

TABLE 34.1: Morphologic Characteristics of the Blast Cells in Romanowsky Stains.

FEATURE	MYELOBLAST	LYMPHOBLAST
1. Size	10-18 μm	10-18 μm
2. Nucleus	Round or oval	Round or oval
3. Nuclear chromatin	Fine meshwork	Slightly clumped
4. Nuclear membrane	Very fine	Fairly dense
5. Nucleoli	2-5	1-2
6. Cytoplasm	Scanty, blue, agranular	Scanty, clear blue, agranular

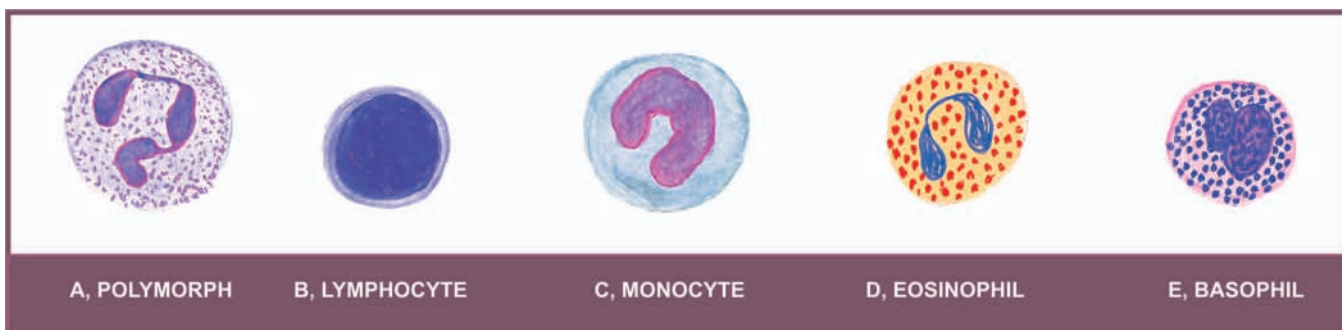


FIGURE 34.3

Morphology of normal mature leucocytes in peripheral blood.

minor degree of diurnal variation with a slight rise in the afternoon. The total white cell count is normally high in pregnancy and following delivery, usually returning to normal within a week. The pathological variations in white cell values together with brief review of their morphology and functions are considered below (Fig. 34.3).

Polymorphs (Neutrophils)

MORPHOLOGY. A polymorphonuclear neutrophil (PMN), commonly called polymorph or neutrophil, is 12–15 μm in diameter. It consists of a characteristic dense nucleus, having 2–5 lobes and pale cytoplasm containing numerous fine violet-pink granules. These granules contain several enzymes and are of 2 types:

Primary or azurophilic granules are large and coarse and appear early at the promyelocyte stage. These granules contain:

i) *Myeloperoxidase (MPO)* used as marker for PMNs (CD13 marker). MPO is involved in formation of hydrogen peroxide which is an important oxygen free radical.

ii) *Hydrolases* such as lysozymes, cathepsin, elastase and proteinase 3. MPO alongwith proteinase 3 is involved in ANCA-mediated vasculitis.

Secondary or specific granules are smaller and more numerous. These appear later at myelocyte stage, are MPO-negative and contain:

- Lysozyme, type IV collagenase, lactoferrin.
- Alkaline phosphatase.
- Plasminogen activator.
- Leucocyte adhesion molecules.

The normal **functions** of neutrophils are as under:

- Chemotaxis* or cell mobilisation in which the cell is attracted towards bacteria or at the site of inflammation.
- Phagocytosis* in which the foreign particulate material is phagocytosed by actively motile neutrophils.
- Killing* of the microorganism is mediated by oxygen-dependent and oxygen-independent pathways (page 100).

PATHOLOGIC VARIATIONS. Pathologic variations in neutrophils include variations in count, morphology and defective function.

Variation in count. An increase in neutrophil count (*neutrophil leucocytosis* or *neutrophilia*) or a decrease in count (*neutropenia*) may occur in various diseases.

Neutrophil leucocytosis. An increase in circulating neutrophils above 7,500/ μl is the commonest type of leucocytosis and occurs most commonly as a response to acute bacterial infections. Some common causes of neutrophilia are as under:

- Acute infections, local or generalised*, especially by cocci but also by certain bacilli, fungi, spirochaetes, parasites and some viruses. For example: pneumonia, cholecystitis, salpingitis, meningitis, diphtheria, plague, peritonitis, appendicitis, actinomycosis, poliomyelitis,

TABLE 34.2: Normal White Blood Cell Counts in Health.

	ABSOLUTE COUNT
TLC	
Adults	4,000–11,000/ μl
Infants (Full term, at birth)	10,000–25,000/ μl
Infants (1 year)	6,000–16,000/ μl
Children (4–7 years)	5,000–15,000/ μl
Children (8–12 years)	4,500–13,500/ μl
DLC IN ADULTS	
Polymorphs (neutrophils) 40–75%	2,000–7,500/ μl
Lymphocytes 20–50%	1,500–4,000/ μl
Monocytes 2–10%	200–800/ μl
Eosinophils 1–6%	40–400/ μl
Basophils <1%	10–100/ μl

abscesses, furuncles, carbuncles, tonsillitis, otitis media, osteomyelitis etc.

2. *Other inflammations* e.g. tissue damage resulting from burns, operations, ischaemic necrosis (such as in MI), gout, collagen-vascular diseases, hypersensitivity reactions etc.

3. *Intoxication* e.g. uraemia, diabetic ketosis, eclampsia, poisonings by chemicals and drugs.

4. *Acute haemorrhage*, internal or external.

5. *Acute haemolysis*.

6. *Disseminated malignancies*.

7. *Myeloproliferative disorders* e.g. myeloid leukaemia, polycythaemia vera, myeloid metaplasia.

8. *Miscellaneous* e.g. following corticosteroid therapy, idiopathic neutrophilia.

Neutropenia. When the absolute neutrophil count falls below 2,500/ μ l, the patient is said to have neutropenia and is prone to develop recurrent infections. Some common causes of neutropenia (and hence leucopenia) are as follows:

1. *Certain infections* e.g. typhoid, paratyphoid, brucellosis, influenza, measles, viral hepatitis, malaria, kala-azar etc.

2. *Overwhelming bacterial infections* especially in patients with poor resistance e.g. miliary tuberculosis, septicaemia.

3. *Drugs, chemicals and physical agents* which induce aplasia of the bone marrow cause neutropenia, e.g. anti-metabolites, nitrogen mustards, benzene, ionising radiation. Occasionally, certain drugs produce neutropenia due to individual sensitivity such as: anti-inflammatory (amidopyrine, phenylbutazone), antibacterial (chlora-

mphenicol, cotrimoxazole), anticonvulsants, anti-thyroids, hypoglycaemics and antihistaminics.

4. *Certain haematological and other diseases* e.g. pernicious anaemia, aplastic anaemia, cirrhosis of the liver with splenomegaly, SLE, Gaucher's disease.

5. *Cachexia and debility*.

6. *Anaphylactoid shock*.

7. *Certain rare hereditary, congenital or familial disorders* e.g. cyclic neutropenia, primary splenic neutropenia, idiopathic benign neutropenia.

VARIATIONS IN MORPHOLOGY. Some of the common variations in neutrophil morphology are shown in Fig. 34.4. These are as under:

1. **Granules.** Heavy, dark staining, coarse toxic granules are characteristic of bacterial infections.

2. **Vacuoles.** In bacterial infections such as in septicaemia, cytoplasmic vacuolation may develop.

3. **Döhle bodies.** These are small, round or oval patches, 2-3 μ m in size, in the cytoplasm. They are mostly seen in bacterial infections.

4. **Nuclear abnormalities.** These include the following:

i) *Sex chromatin* is a normal finding in 2-3% of neutrophils in female sex. It consists of a drumstick appendage of chromatin, about 1 μ m across, and attached to one of the nuclear lobes by a thin chromatin strand. Their presence in more than 20% of PMNs is indicative of female sex chromosomes (page 546).

ii) A '*shift-to-left*' is the term used for appearance of neutrophils with decreased number of nuclear lobes in the peripheral blood e.g. presence of band and stab forms and a few myelocytes in the peripheral blood. It is seen in severe infections, leucoerythroblastic reaction or leukaemia.

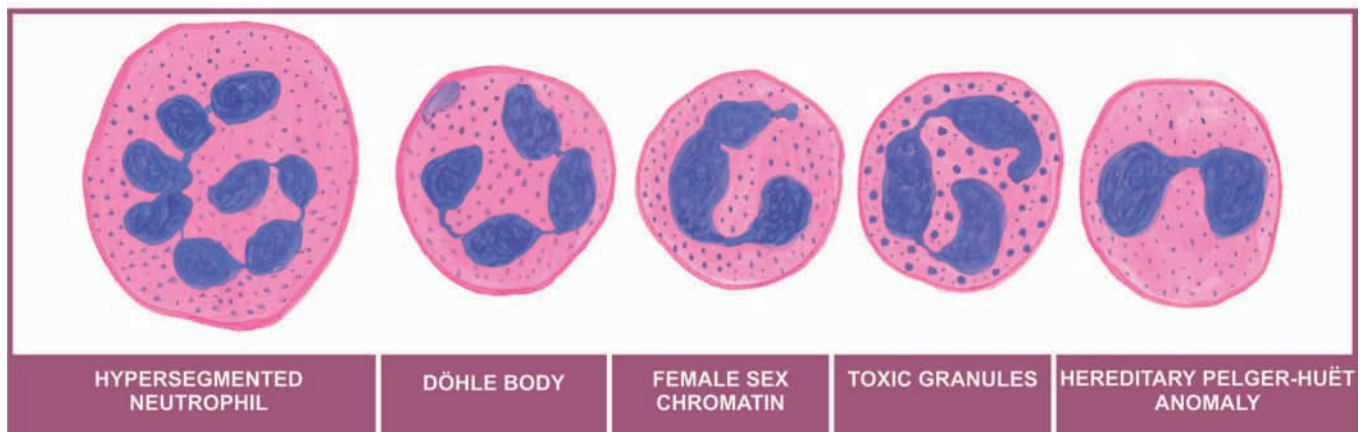


FIGURE 34.4

Common variations in neutrophil morphology.

iii) A 'shift-to-right' is appearance of hypersegmented (more than 5 nuclear lobes) neutrophils in the peripheral blood such as in megaloblastic anaemia, uraemia, and sometimes in leukaemia.

iv) *Pelger-Huët anomaly* is an inherited disorder in which majority of neutrophils have decreased number of nuclear segments (1-2) and coarsely staining chromatin. The nuclei appear rod-like, dumb-bell or spectacle-like.

DEFECTIVE FUNCTIONS. The following abnormalities in neutrophil function may sometimes be found:

1. **Defective chemotaxis** e.g. in a rare congenital abnormality called lazy-leucocyte syndrome; following corticosteroid therapy, aspirin ingestion, alcoholism, and in myeloid leukaemia.
2. **Defective phagocytosis** due to lack of opsonisation e.g. in hypogammaglobulinaemia, hypocomplementaemia, after splenectomy, in sickle cell disease.
3. **Defective killing** e.g. in chronic granulomatous disease, Chédiak-Higashi syndrome, myeloid leukemias.

Lymphocytes

MORPHOLOGY. Majority of lymphocytes in the peripheral blood are *small* (9-12 μm in diameter) but *large* lymphocytes (12-16 μm in diameter) are also found. Both small and large lymphocytes have round or slightly indented nucleus with coarsely-clumped chromatin and scanty basophilic cytoplasm. Plasma cells are derived from B lymphocytes under the influence of appropriate stimuli. The nucleus of plasma cell is eccentric and has cart-wheel pattern of clumped nuclear chromatin. The cytoplasm is characteristically deeply basophilic with a pale perinuclear zone. Plasma cells are normally not present in peripheral blood but their pathological proliferation occurs in myelomatosis. Reactive lymphocytes (or Turk cells or plasmacytoid lymphocytes) are seen in certain viral infections and have sufficiently basophilic cytoplasm that they resemble plasma cells.

Functionally, there are 3 types of lymphocytes and possess distinct surface markers called clusters of differentiation (CD) which aid in identification of stage of their differentiation:

T lymphocytes i.e. thymus-dependent lymphocytes, which mature in the thymus and are also known as thymocytes. They are mainly involved in direct action on antigens and are therefore involved in *cell-mediated immune* (CMI) reaction by its subsets such as cytotoxic (killer) T cells (CD3+), CD8+ T cells, and *delayed hypersensitivity reaction* by CD4+ T cells.

B lymphocytes i.e. bone marrow-dependent or bursa-equivalent lymphocytes as well as their derivatives,

plasma cells, are the source of specific immunoglobulin antibodies. They are, therefore, involved in *humoral immunity* (HI) or *circulating immune reactions*.

NK cells i.e. natural killer cells are those lymphocytes which morphologically have appearance of lymphocytes but do not possess functional features of T or B cells. As the name indicates they are identified with 'natural' or innate immunity and bring about direct 'killing' of microorganisms or lysis of foreign body.

PATHOLOGIC VARIATIONS. A rise in the absolute count of lymphocytes exceeding the upper limit of normal (above 4,000/ μm) is termed *lymphocytosis*, while absolute lymphocyte count below 1,500/ μm is referred to as *lymphopenia*.

Lymphocytosis. Some of the common causes of lymphocytosis are as under:

1. *Certain acute infections* e.g. pertussis, infectious mononucleosis, viral hepatitis, infectious lymphocytosis.
2. *Certain chronic infections* e.g. brucellosis, tuberculosis, secondary syphilis.
3. *Haematopoietic disorders* e.g. lymphocytic leukaemias, lymphoma, heavy chain disease.
4. *Relative lymphocytosis* is found in viral exanthemas, convalescence from acute infections, thyrotoxicosis, conditions causing neutropenia.

Lymphopenia. Lymphopenia is uncommon and occurs in the following conditions:

1. Most acute infections.
2. Severe bone marrow failure.
3. Corticosteroid and immunosuppressive therapy.
4. Widespread irradiation.

Monocytes

MORPHOLOGY. The monocyte is the largest mature leucocyte in the peripheral blood measuring 12-20 μm in diameter. It possesses a large, central, oval, notched or indented or horseshoe-shaped nucleus which has characteristically fine reticulated chromatin network. The cytoplasm is abundant, pale blue and contains many fine granules and vacuoles.

The main **functions** of monocytes are as under:

1. *Phagocytosis* of antigenic material or microorganisms.
2. Immunologic function as *antigen-presenting cells* and present the antigen to lymphocytes to deal with further.
3. As *mediator of inflammation*, they are involved in release of prostaglandins, stimulation of the liver to secrete acute phase reactants.

Tissue macrophages of different types included in RE system are derived from blood monocytes (page 109).

PATHOLOGIC VARIATIONS. A rise in the blood monocytes above 800/ml is termed *monocytosis*. Some common causes of monocytosis are as follows:

1. *Certain bacterial infections* e.g. tuberculosis, subacute bacterial endocarditis, syphilis.
2. *Viral infections.*
3. *Protozoal and rickettsial infections* e.g. malaria, typhus, trypanosomiasis, kala-azar.
4. *Convalescence from acute infection.*
5. *Haematopoietic disorders* e.g. monocytic leukaemia, lymphomas, myeloproliferative disorders, multiple myeloma, lipid storage disease.
6. *Malignancies* e.g. cancer of the ovary, stomach, breast.
7. *Granulomatous diseases* e.g. sarcoidosis, inflammatory bowel disease.
8. *Collagen-vascular diseases.*

Eosinophils

MORPHOLOGY. Eosinophils are similar to segmented neutrophils in size (12-15 μm in diameter), and have coarse, deep red staining granules in the cytoplasm and have usually two nuclear lobes. Granules in eosinophils contain basic protein and stain more intensely for peroxidase than granules in the neutrophils. In addition, eosinophils also contain cell adhesion molecules, cytokines (IL-3, IL-5), and a protein that precipitates Charcot-Leyden crystals in lung tissues in asthmatic patients.

Eosinophils are involved in reactions to foreign proteins and to antigen-antibody reactions.

PATHOLOGIC VARIATIONS. An increase in the number of eosinophilic leucocytes above 400/ μl is referred to as *eosinophilia* and below 40/ μl is termed as *eosinopenia*.

Eosinophilia. The causes are:

1. Allergic disorders e.g. bronchial asthma, urticaria, angioneurotic oedema, hay fever, drug hypersensitivity.
2. *Parasitic infestations* e.g. trichinosis, echinococcosis, intestinal parasitism.
3. *Skin diseases* e.g. pemphigus, dermatitis herpetiformis, erythema multiforme.
4. *Löffler's syndrome.*
5. *Pulmonary infiltration with eosinophilia* (PIE) syndrome.
6. *Tropical eosinophilia.*
7. *Haematopoietic diseases* e.g. CML, polycythaemia vera, pernicious anaemia, Hodgkin's disease, following splenectomy.

8. *Malignant diseases* with metastases.
9. *Irradiation.*
10. *Miscellaneous disorders* e.g. polyarteritis nodosa, rheumatoid arthritis, sarcoidosis.

Eosinopenia. Adrenal steroids and ACTH induce eosinopenia in man.

Basophils

MORPHOLOGY. Basophils resemble the other mature granulocytes but are distinguished by coarse, intensely basophilic granules which usually fill the cytoplasm and often overlie and obscure the nucleus.

The granules of basophils contain heparin, histamine and 5-HT. In the tissues these cells become *mast cells*. Mast cells or basophils on degranulation are associated with histamine release.

PATHOLOGIC VARIATIONS. Basophil leucocytosis or *basophilia* refers to an increase in the number of basophilic leucocytes above 100/ μl . Basophilia is unusual and is found in the following conditions:

1. Chronic myeloid leukaemia
2. Polycythaemia vera
3. Myelosclerosis
4. Myxoedema
5. Ulcerative colitis
6. Following splenectomy
7. Hodgkin's disease
8. Urticaria pigmentosa.

INFECTIOUS MONONUCLEOSIS

Infectious mononucleosis (IM) or glandular fever is a benign, self-limiting lymphoproliferative disease caused by Epstein-Barr virus (EBV), one of the herpesviruses. Infection may occur from childhood to old age but the classical acute infection is more common in teenagers and young adults. The infection is transmitted by person-to-person contact such as by kissing with transfer of virally-contaminated saliva. Groups of cases occur particularly in young people living together in boarding schools, colleges, camps and military institutions. Primary infection in childhood is generally asymptomatic, while 50% of adults develop clinical manifestations. The condition is so common that by the age of 40, most people have been infected and developed antibodies. It may be mentioned here that EBV is oncogenic as well and is strongly implicated in the African (endemic) Burkitt's lymphoma and nasopharyngeal carcinoma as discussed on page 266.

Pathogenesis

EBV, the etiologic agent for IM, is a B lymphotropic herpesvirus. The disease is characterised by fever, generalised lymphadenopathy, hepatosplenomegaly, sore throat, and appearance in blood of atypical 'mononucleosis cells'. The pathogenesis of these pathologic features is outlined below (Fig. 34.5):

1. In a susceptible sero-negative host who lacks antibodies, the virus in the contaminated saliva *invades and replicates within epithelial cells* of the salivary gland and then enters B cells in the lymphoid tissues which possess receptors for EBV. The infection spreads throughout the body via the bloodstream or by infected B cells.
2. Viraemia and death of infected B cells causes an acute febrile illness and appearance of specific humoral antibodies which peak about 2 weeks after the infection and persist throughout life. The appearance of antibodies marks the *disappearance of virus from the blood*.
3. Though the viral agent has disappeared from the blood, the EBV-infected B cells continue to be present in the circulation as latent infection. *EBV-infected B cells undergo polyclonal activation and proliferation*. These cells perform two important roles which are the characteristic diagnostic features of IM:

- i) They secrete *antibodies*—initially IgM but later IgG appears. IgM antibody is the heterophile anti-sheep antibody used for diagnosis of IM while IgG antibody persists for life and provides immunity against re-infection.
 - ii) They *activate T lymphocytes* of two types— CD8+ T lymphocytes and cytotoxic (suppressor) T cells. CD8+ T cells bring about killing of B cells while the cytotoxic T cells are pathognomonic atypical lymphocytes seen in blood in IM.
4. The proliferation of these cells is responsible for *generalised lymphadenopathy and hepatosplenomegaly*.
 5. The *sore throat* in IM may be caused by either necrosis of B cells or due to viral replication within the salivary epithelial cells in early stage.
- Besides the involvement of EBV in the pathogenesis of IM, its role in neoplastic transformation in nasopharyngeal carcinoma and Burkitt's lymphoma is discussed in Chapter 21 and diagrammatically depicted in Fig. 34.5.

Clinical Features

The incubation period of IM is 30-50 days in young adults, while children have shorter incubation period.

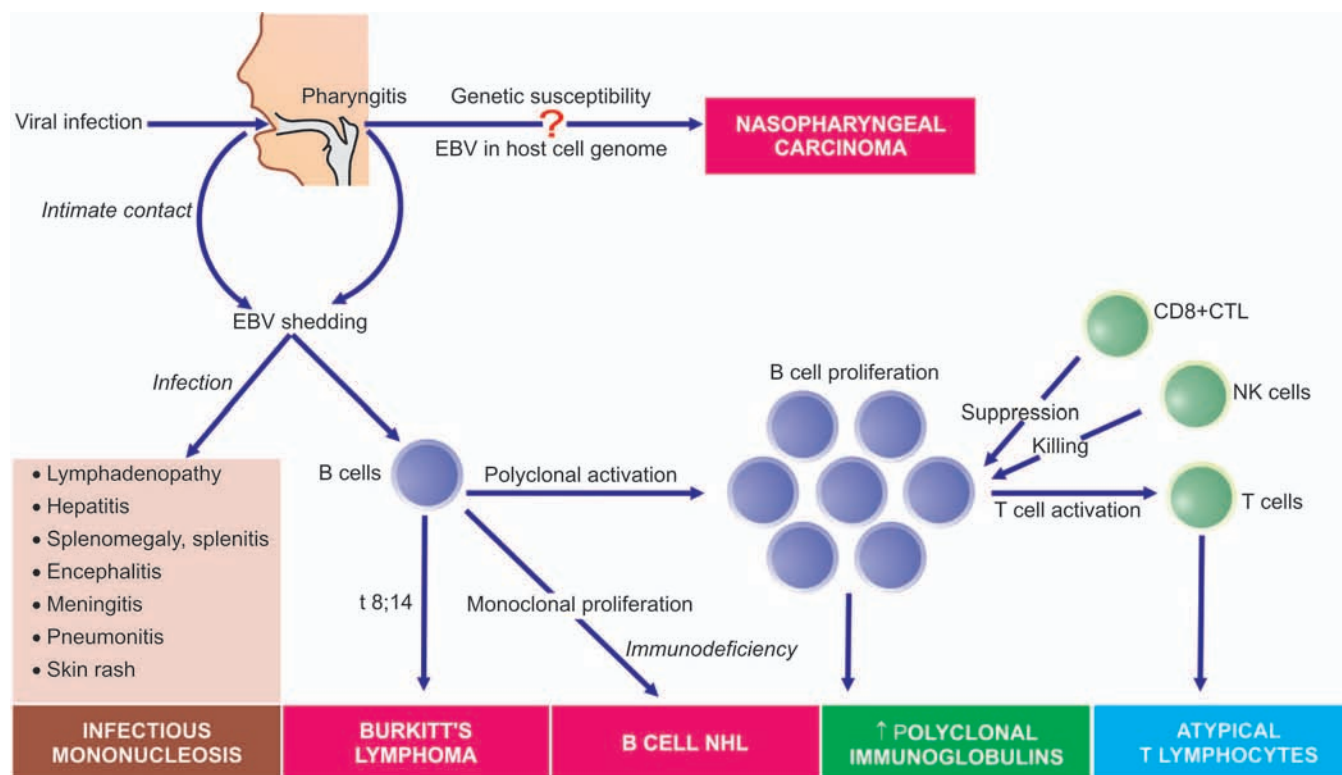


FIGURE 34.5

The role of EBV in the pathogenesis of infectious mononucleosis, nasopharyngeal carcinoma and Burkitt's lymphoma.

A prodromal period of 3-5 days is followed by frank clinical features lasting for 1-3 weeks, and complete recovery after 2 months. The usual clinical features are as under:

1. During prodromal period (first 3-5 days), the symptoms are mild such as malaise, myalgia, headache and fatigue.

2. Frank clinical features (next 7-21 days), commonly are fever, sore throat and bilateral cervical lymphadenopathy. Less commonly, splenomegaly (50% patients), hepatomegaly (10% cases), transient erythematous maculopapular eruption on the trunk and extremities, and neurologic manifestations are found. Pneumonia and cardiac involvement are infrequent. One of the complications of IM is splenic rupture due to splenitis.

Laboratory Findings

The diagnosis of IM is made by characteristic haematologic and serologic findings.

1. Blood picture. The peripheral blood shows a moderate rise in total white cell count (10,000-20,000/ μ l) with an absolute lymphocytosis. The lymphocytosis is due to rise in normal as well as atypical T lymphocytes. Essential to the diagnosis of IM is the presence of at least 10-12% *atypical T cells (or mononucleosis cells)* of the total lymphocytes in the peripheral blood. The mononucleosis cells are variable in appearance and are classed as Downey type I, II and III, of which Downey type I are found most frequently. These atypical T lymphocytes are usually of the size of large lymphocytes (12-16 μ m diameter). The nucleus, rather than the usual round configuration, is oval, kidney-shaped or slightly lobate and contains relatively fine chromatin without nucleoli suggesting an immature pattern but short of leukaemic features. The cytoplasm is more abundant, basophilic and finely granular. The greatest number of atypical lymphocytes is found between 7th to 10th day of the illness and these cells may persist in the blood for up to 2 months.

2. Serologic diagnosis. The second characteristic laboratory finding is the demonstration of antibodies in the serum of infected patient. These are as under:

i) *Heterophile antibodies* against sheep red cells are found in the serum at high titer (Paul-Bunnell test) in 80-90% patients of IM, with a peak during the 2nd and 3rd week. Similar antibody is also produced in patients suffering from serum sickness and has to be distinguished by differential absorption studies. Heterophile antibody against ox and horse is also produced and is more specific. Heterophile antibody

in the early stage is IgM but later IgA class antibody appears which persists throughout life.

ii) *Specific antibody for EBV antigen.* The appearance of specific EBV antibody in the first 2-3 weeks can also be demonstrated.

iii) *EBV antigen.* If viral diagnostic facilities are available, EBV antigen can also be demonstrated.

3. Other laboratory findings. In addition, abnormalities of the liver function test may be found in some cases. These include elevated serum levels of transaminases (SGOT and SGPT), rise in serum alkaline phosphatase and mild icterus.

LEUKAEMOID REACTIONS

Leukaemoid reaction is defined as a reactive excessive leucocytosis in the peripheral blood resembling that of leukaemia in a subject who does not have leukaemia. In spite of confusing blood picture, the clinical features of leukaemia such as splenomegaly, lymphadenopathy and haemorrhages are usually absent and the features of underlying disorder causing the leukaemoid reaction are generally obvious.

Leukaemoid reaction may be myeloid or lymphoid; the former is much more common.

MYELOID LEUKAEMOID REACTION

CAUSES. The majority of leukaemoid reactions involve the granulocyte series. It may occur in association with a wide variety of diseases. These are as under:

1. *Infections* e.g. staphylococcal pneumonia, disseminated tuberculosis, meningitis, diphtheria, sepsis, endocarditis, plague, infected abortions etc.
2. *Intoxication* e.g. eclampsia, mercury poisoning, severe burns.
3. *Malignant diseases* e.g. multiple myeloma, myelofibrosis, Hodgkin's disease, bone metastases.
4. *Severe haemorrhage and severe haemolysis.*

LABORATORY FINDINGS. Myeloid leukaemoid reaction is characterised by the following laboratory features:

1. *Leucocytosis*, usually moderate, not exceeding 100,000/ μ l.
2. Proportion of *immature cells* mild to moderate, comprised by metamyelocytes, myelocytes (5-15%), and blasts fewer than 5% i.e. the blood picture simulates that of CML.
3. Infective cases may show *toxic granulation and Döhle bodies* in the cytoplasm of neutrophils.

4. *Neutrophil alkaline phosphatase (NAP) score* in the cytoplasm of mature neutrophils in leukaemoid reaction is characteristically high and is very useful to distinguish it from chronic myeloid leukaemia in doubtful cases.

5. *Cytogenetic studies* may be helpful in exceptional cases which reveal negative Philadelphia chromosome in myeloid leukaemoid reaction but positive in cases of CML.

6. *Additional features* include anaemia, normal-to-raised platelet count, myeloid hyperplasia of the marrow and absence of infiltration by immature cells in organs and tissues.

LYMPHOID LEUKAEMOID REACTION

CAUSES. Lymphoid leukaemoid reaction may be found in the following conditions:

1. *Infections* e.g. infectious mononucleosis, cytomegalovirus infection, pertussis (whooping cough), chickenpox, measles, infectious lymphocytosis, tuberculosis.
2. *Malignant diseases* may rarely produce lymphoid leukaemoid reaction.

LABORATORY FINDINGS. The blood picture is characterised by the following findings:

1. Leucocytosis not exceeding 100,000/ μ l.
2. The differential white cell count reveals mostly mature lymphocytes simulating the blood picture found in cases of CLL.

LEUKAEMIAS

DEFINITION AND CLASSIFICATION

The leukaemias are a group of disorders characterised by malignant transformation of blood-forming cells. The proliferation of leukaemic cells takes place primarily in the bone marrow, and in certain forms, in the lymphoid tissues. Ultimately, the abnormal cells appear in the peripheral blood raising the total white cell count to high level. In addition, features of bone marrow failure (e.g. anaemia, thrombocytopenia, neutropenia) and involvement of other organs (e.g. liver, spleen, lymph nodes, meninges, brain, skin etc) occur.

In general, leukaemias are classified on the basis of cell types predominantly involved, into *myeloid* and *lymphoid*, and on the basis of natural history of the disease, into *acute* and *chronic*. Thus, the main types are: *acute myeloblastic leukaemia* and *acute lymphoblastic leukaemia* (AML and ALL), and *chronic myeloid leukaemia* and *chronic lymphocytic leukaemias* (CML and CLL); *hairy*

cell leukaemia (HCL) is an unusual variant of lymphoid neoplasia.

Leukaemias account for 4% of all cancer deaths. Generally, acute leukaemias have a rapidly downhill course, whereas chronic leukaemias tend to have more indolent behaviour. The incidence of both acute and chronic leukaemias is higher in men than in women. ALL is primarily a disease of children and young adults, whereas AML occurs at all ages. CLL tends to occur in the elderly, while CML is found in middle age.

ETIOLOGY

The etiology of leukaemia is not known in most patients. However, a number of factors have been implicated.

1. GENETIC FACTORS. There is high concordance rate among identical twins if acute leukaemia develops in the first year of life. Families with excessive incidence of leukaemia have been identified. Acute leukaemia occurs with increased frequency with a variety of congenital disorders such as Down's, Bloom's, Klinefelter's and Wiskott-Aldrich's syndromes, Fanconi's anaemia and ataxia telangiectasia.

2. ENVIRONMENTAL FACTORS. Certain environmental factors are known to play a role in the etiology of leukaemia. These include the following:

i) **Ionising radiation** e.g. in individuals exposed to occupational radiation exposure, patients receiving radiation therapy, and Japanese survivors of the atomic bomb explosions. Radiation exposure is related to the development of CML, AML and ALL *but not to CLL or HCL*.

ii) **Chemical carcinogens** e.g. benzene and other aromatic hydrocarbons are associated with the development of AML.

iii) **Certain drugs** e.g. treatment with alkylating agents and other chemotherapeutic agents is associated with increased incidence of AML.

3. INFECTION. Induction of leukaemias in experimental animals by RNA viruses (retroviruses) has been studied for quite sometime but more recently viral etiology of adult T cell leukaemia-lymphoma (ATLL) by a human retrovirus called human T cell leukaemia-lymphoma virus I (HTLV-I) and HTLV II for T cell variant of hairy cell leukaemia has been established (Chapter 21).

PATHOGENESIS (LEUKAEMOGENESIS)

The leukaemia arises following *malignant transformation of a single clone of cells belonging to myeloid or lymphoid*

series, followed by proliferation of the transformed clone. The evolution of leukaemia is multi-step process, and in many cases, acute leukaemia may develop after a pre-existing myelodysplastic or myeloproliferative disorder.

The salient features in the pathogenesis of leukaemias are as under:

- In acute leukaemia, the single most prominent characteristic of the leukaemic cells is a defect in maturation beyond the myeloblast or promyelocyte level in AML, and the lymphoblast level in ALL. However, it may be emphasised here that it is the *maturation defect* in leukaemic blasts rather than rapid proliferation of leukaemic cells responsible for causing acute leukaemia. In fact, the generation time of leukaemic blasts is somewhat prolonged rather than shortened.

- The leukaemic cells proliferate primarily in the bone marrow, circulate in the blood and *infiltrate into other tissues* such as lymph nodes, liver, spleen, skin, viscera and the central nervous system.

- The *mechanism of leukaemic transformation* is poorly understood. But the basic defect lies in the DNA, conferring a heritable malignant characteristic to the transformed cell and its progeny. A neoplastic phenotype may be induced by RNA viruses (e.g. HTLV-I) and causes insertional mutagenesis for which oncogenes may play a role as discussed in Chapter 21. A number of clonal cytogenetic abnormalities have been reported in association with the various forms of acute and chronic leukaemias. The most consistent chromosomal abnormality among these is Philadelphia (Ph) chromosome seen in 70-90% cases with CML, involving reciprocal translocation of parts of long arm of chromosome 22 to the long arm of chromosome 9 i.e. t(9;22) (Fig. 34.6).

- As the leukaemic cells accumulate in the bone marrow, they *suppress normal haematopoietic stem cells*, partly by physically replacing the normal marrow precursors. However, some patients with acute leukaemia have a hypocellular marrow indicating that marrow failure is not simply due to overcrowding by leukaemic cells but may inhibit normal haematopoiesis via cell-mediated or humoral mechanisms. Nevertheless, some normal haematopoietic stem cells do remain in the marrow which are capable of proliferating and restoring normal haematopoiesis after effective anti-leukaemic treatment.

ACUTE LEUKAEMIAS

Acute leukaemias are characterised by predominance of undifferentiated leucocyte precursors or leukaemic blasts. Acute leukaemias may be derived from the myeloid stem cells called *acute myeloblastic leukaemia*

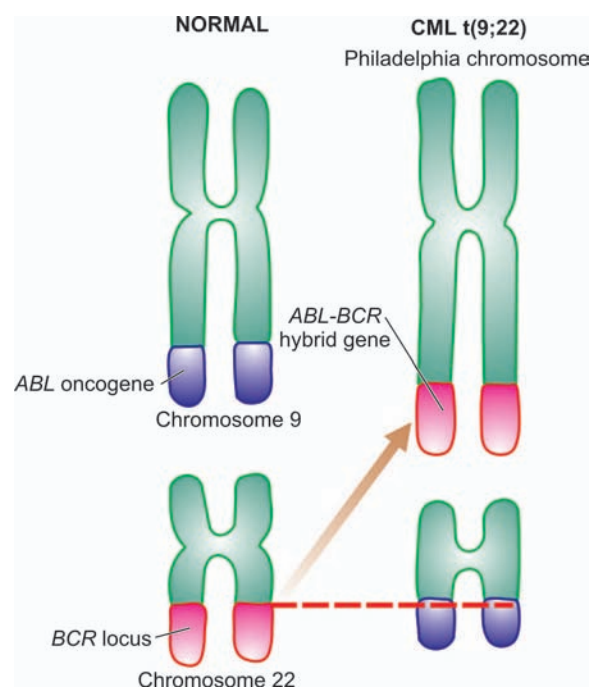


FIGURE 34.6

The Philadelphia (Ph) chromosome. There is reciprocal translocation of the part of the long arms of chromosome 22 to the long arms of chromosome 9 written as t(9;22).

(AML), or from the lymphoid stem cells termed *acute lymphoblastic leukaemia (ALL)*.

Recently, more definite criteria for diagnosis and classification of acute leukaemias have been laid down by a group of French, American and British haematologists commonly called the *FAB classification*. According to this system, a leukaemia is acute if the bone marrow consists of more than 30% blasts. FAB classification divides AML into 8 subtypes (M0 to M7) and ALL into 3 subtypes (L1 to L3) as shown in Table 34.3. Another classification called *European-American classification of Lymphoid Neoplasms (REAL classification)* has been formulated and includes lymphoid leukaemias (acute and chronic) and non-Hodgkin's lymphomas since they represent the counterparts of neoplastic cells in the bone marrow and lymphoid tissue (page 526). More recently, WHO classification for AML has been proposed which takes in to consideration cytogenetic and molecular abnormalities in different types.

Thus, the basis of these categorisation of leukaemias can be summed up as:

- *FAB classification* is based on *morphologic and cytochemical features*.
- *REAL classification* is based on *immunophenotypic features*.

■ WHO classification is based on cytogenetic and molecular features.

Clinical Features

AML and ALL share many clinical features. In approximately 25% of patients with AML, a preleukaemic syndrome with anaemia and other cytopenias is usually present for a few months to years prior to the development of overt leukaemia.

Clinical manifestations of acute leukaemias are divided into 2 groups: those due to bone marrow failure, and those due to organ infiltration.

I. DUE TO BONE MARROW FAILURE. These are as under:

1. *Anaemia* producing pallor, lethargy, dyspnoea.
2. *Bleeding manifestations* due to thrombocytopenia causing spontaneous bruises, petechiae, bleeding from gums and other bleeding tendencies.
3. *Infections* are quite common and include those of mouth, throat, skin, respiratory, perianal and other sites.
4. *Fever* is generally attributed to infections in acute leukaemia but sometimes no obvious source of infection can be found and may occur in the absence of infection.

II. DUE TO ORGAN INFILTRATION. The clinical manifestations of acute leukaemia are more often due to replacement of the marrow and other tissues by leukaemic cells. These features are as under:

1. *Pain and tenderness of bones* (e.g. sternal tenderness) are due to bone infarcts or subperiosteal infiltrates by leukaemic cells. These features are more frequent in children with ALL.
2. *Lymphadenopathy* and enlargement of the *tonsils* are more common in ALL.
3. *Splenomegaly* of moderate grade is common, especially in ALL. Splenic infarction, subcapsular haemorrhages, and rarely, splenic rupture may occur.
4. *Hepatomegaly* is frequently present due to leukaemic infiltration but the infiltrates usually do not interfere with the function of the liver.
5. *Leukaemic infiltration of the kidney* may be present and ordinarily does not interfere with its function unless secondary complications such as haemorrhage or blockage of ureter supervene.
6. *Gum hypertrophy* due to leukaemic infiltration of the gingivae is a frequent finding in myelomonocytic (M4) and monocytic (M5) leukaemias.
7. *Chloroma or granulocytic sarcoma* is a localised tumour-forming mass occurring in the skin or orbit due to local

infiltration of the tissues by leukaemic cells. The tumour is greenish in appearance due to the presence of myeloperoxidase.

8. *Meningeal involvement* manifested by raised intracranial pressure, headache, nausea and vomiting, blurring of vision and diplopia are seen more frequently in ALL during haematologic remission. Sudden death from massive intracranial haemorrhage as a result of leucostasis may occur.

9. *Other organ infiltrations* include testicular swelling in ALL and mediastinal compression in T cell type ALL.

Laboratory Findings

The diagnosis of acute leukaemia is made by a combination of routine blood picture and bone marrow examination, coupled with cytochemical stains and certain biochemical investigations.

I. BLOOD PICTURE. Findings of routine haematologic investigations are as under (Fig. 34.7) (COLOUR PLATE X: CL 40):

1. Anaemia. Anaemia is almost always present in acute leukaemias. It is generally severe, progressive and normochromic in type. A moderate reticulocytosis up to 5% and a few nucleated red cells may be present.

2. Thrombocytopenia. The platelet count is usually moderately to severely decreased (below 50,000/ μ l) but occasionally it may be normal. Bleeding

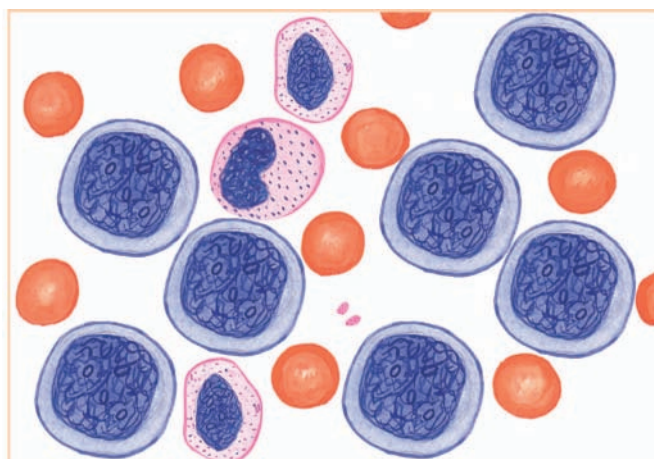


FIGURE 34.7

PBF findings in a case of acute myeloblastic leukaemia (AML).

tendencies in acute leukaemia are usually correlated with the level of thrombocytopenia but most serious spontaneous haemorrhagic episodes develop in patients with fewer than 20,000/ μ l platelets. Acute promyelocytic leukaemia (M3) may be associated with a serious coagulation abnormality called disseminated intravascular coagulation (DIC).

3. White blood cells. The total WBC count ranges from subnormal-to-markedly elevated values. In 25% of patients, the total WBC count at presentation is reduced to 1,000-4,000 / μ l. More often, however, there is progressive rise in white cell count which may exceed 100,000/ μ l in more advanced disease. The majority of leucocytes in the peripheral blood are blasts and there is often neutropenia due to marrow infiltration by leukaemic cells. Some patients present with pancytopenia and have a few blasts (*sub-leukaemic leukaemia*) or no blasts (*aleukaemic leukaemia*) in the blood. Both these conditions are nowadays included under '*myelodysplastic syndrome*' (page 480). The basic morphologic features of myeloblasts, lymphoblasts and monoblasts are described in Table 34.1. Typical characteristics of different forms of AML (M0 to M7) and ALL (L1 to L3) are given in Table 34.3. In some instances, the *identification of blast cells is greatly aided by the company they keep* i.e. by more mature and easily identifiable leucocytes in the company of blastic cells of myeloid or lymphoid series. It is usual to find some '*smear cells*' in the peripheral blood which represent degenerated leucocytes, particularly in the case of ALL.

II. BONE MARROW EXAMINATION. An examination of bone marrow aspirate or trephine reveals the following features:

1. Cellularity. Typically, the marrow is hypercellular but sometimes a 'blood tap' or 'dry tap' occurs. A dry tap in acute leukaemia may be due to pancytopenia, but sometimes even when the marrow is filled with leukaemic cells they cannot be aspirated because the cells are adhesive and enmeshed in reticulin fibres. In such cases, trephine biopsy should be done.

2. Leukaemic cells. The bone marrow is generally tightly packed with leukaemic blast cells. The diagnosis of the type of leukaemic cells, according to FAB classification, is generally possible with routine Romanowsky stains but cytochemical stains may be employed as an adjunct to Romanowsky staining for determining the type of leukaemia. The essential criteria for diagnosis of acute leukaemia, as per FAB

classification, is the presence of at least 30% blasts in the bone marrow. However, lately As per WHO classification of leukaemias, these criteria have been revised upwards to 20% blasts in the marrow for labelling and treating a case as AML.

3. Erythropoiesis. Erythropoietic cells are reduced. Dyserythropoiesis, megaloblastic features and ring sideroblasts are commonly present.

4. Megakaryocytes. They are usually reduced or absent.

5. Cytogenetics. Chromosomal analysis of dividing leukaemic cells in the marrow shows karyotypic abnormalities in 75% of cases which may have a relationship to prognosis. WHO classification has recently emphasised on the categorisation of acute leukaemias on the basis of cytogenetic abnormalities. Two of the most consistent cytogenetic abnormalities in specific FAB groups are as under:

i) M3 cases have t(15;17)(q22;q12).

ii) M4Eo (E for abnormal eosinophils in the bone marrow) cases have inv(16)(p13q22).

III. CYTOCHEMISTRY. Some of the commonly employed cytochemical stains, as an aid to classify the type of acute leukaemia, are listed below (also see Table 34.3):

1. Myeloperoxidase: Positive in immature myeloid cells containing granules and Auer rods but negative in M0 myeloblasts.

2. Sudan Black: Positive in immature cells in AML.

3. Periodic acid-Schiff (PAS): Positive in immature lymphoid cells and in erythroleukaemia (M6).

4. Non-specific esterase (NSE): Positive in monocytic series (M4 and M5).

5. Acid phosphatase: Focal positivity in leukaemic blasts in ALL and diffuse reaction in monocytic cells (M4 and M5).

IV. IMMUNOPHENOTYPING. AML cells express CD13 and CD33 antigens; M7 shows CD41 and CD42 positivity. However, for ALL the FAB classification given in Table 34.3 is based on morphologic features, currently as per REAL-WHO classification for all lymphoid malignancies (lymphoid leukaemias and lymphomas), lymphoid leukaemias (both ALL and CLL) are subdivided on the basis of immunologic features by identification as regards their B or T cell origin and cytogenetic abnormalities (*see* Table 34.4, page 495). According to this classification, ALL is redivided into pre-B ALL, T cell ALL and B cell ALL

TABLE 34.3: Revised FAB Classification of Acute Leukaemias.

FAB CLASS	PER CENT CASES	MORPHOLOGY	CYTOCHEMISTRY
ACUTE MYELOBLASTIC LEUKAEMIA (AML)			
M0: <i>Minimally differentiated AML</i>	2	Blasts lack definite cytologic and cytochemical features but have myeloid lineage antigens	Myeloperoxidase –
M1: <i>AML without maturation</i>	20	Myeloblasts predominate; few if any granules or Auer rods	Myeloperoxidase +
M2: <i>AML with maturation</i>	30	Myeloblasts with promyelocytes predominate; Auer rods may be present	Myeloperoxidase +++
M3: <i>Acute promyelocytic leukaemia</i>	5	Hypergranular promyelocytes; often with multiple Auer rods per cell	Myeloperoxidase +++
M4: <i>Acute myelomonocytic leukaemia (Naegeli type)</i>	30	Mature cells of both myeloid and monocytic series in peripheral blood; myeloid cells resemble M2	Myeloperoxidase ++ Non-specific esterase +
M5: <i>Acute monocytic leukaemia (Schilling type)</i>	10	Two subtypes: M5a shows poorly-differentiated monoblasts, M5b shows differentiated promonocytes and monocytes	Non-specific esterase ++
M6: <i>Acute erythroleukaemia (Di Guglielmo's syndrome)</i>	<5	Erythroblasts predominate (>50%); myeloblasts and promyelocytes also increased	Erythroblasts: PAS + Myeloblasts: myeloperoxidase +
M7: <i>Acute megakaryocytic leukaemia</i>	<5	Pleomorphic undifferentiated blasts predominate; react with antiplatelet antibodies	Platelet peroxidase +
ACUTE LYMPHOBLASTIC LEUKAEMIA (ALL)			
L1: <i>Childhood-ALL (B-ALL, and T-ALL)</i>	More common in children	Homogeneous small lymphoblasts; scanty cytoplasm, regular round nuclei, inconspicuous nucleoli	PAS ± Acid phosphatase ±
L2: <i>Adult-ALL (mostly T-ALL)</i>	More frequent in adults	Heterogeneous lymphoblasts; variable amount of cytoplasm, irregular or cleft nuclei, large nucleoli	PAS ± Acid phosphatase ±
L3: <i>Burkitt type-ALL (B-ALL)</i>	Uncommon	Large homogeneous lymphoblasts; round nuclei, prominent nucleoli, cytoplasmic vacuolation	PAS – Acid phosphatase –

with distinct cytogenetic and immunologic features (Table 34.4).

V. OTHER INVESTIGATIONS. Other laboratory tests which may be of some help in classifying leukaemias are as under:

1. Serum muramidase. Serum levels of lysozyme (i.e. muramidase) are elevated in myelomonocytic (M4) and monocytic (M5) leukaemias.

2. Serum uric acid. Because of rapidly growing number of leukaemic cells, serum uric acid level is frequently increased. The levels are further raised after treatment with cytotoxic drugs because of increased cell breakdown.

The salient differences between the two main forms of acute leukaemia are summarised in Table 34.5.

CHRONIC LEUKAEMIAS

Chronic leukaemias are those haematologic malignancies in which the predominant leukaemic cells are

initially well-differentiated and easily recognisable as regards their cell type. Chronic leukaemias are divided into 2 main types: chronic myeloid (granulocytic) leukaemia (CML or CGL), and chronic lymphocytic leukaemia (CLL). Less common variants include: chronic eosinophilic, chronic basophilic, chronic monocytic, chronic neutrophilic and chronic lymphosarcoma cell leukaemias. An unusual chronic lymphoproliferative variant is hairy cell leukaemia (HCL). In general, chronic leukaemias have a better prognosis than the acute leukaemias. Currently CML has come to be classified alongwith other myeloproliferative syndromes due to common histogenesis from haematopoietic stem cells (described later). However, for the purpose of present discussion, CML is described here alongwith other chronic leukaemias.

CHRONIC MYELOID LEUKAEMIA (CML)

Chronic myeloid (myelogenous, granulocytic) leukaemia comprises about 20% of all leukaemias and its peak incidence is seen in 3rd and 4th decades of life. A

TABLE 34.4: WHO-REAL Classification of ALL on the Basis of Immunologic and Cytogenetic Features.

SUBTYPE	INCIDENCE	MARKERS	FAB SUBTYPE	CYTOGENETIC ABNORMALITIES
<i>Pre-B ALL</i>	75%	CD10+ (90%), TdT+	L1, L2	t(9;22) i.e. Philadelphia+ALL
<i>B cell ALL</i>	5%	CD10+ (50%), TdT-	L3	t(8;14) (Burkitt's leukaemia)
<i>T cell ALL</i>	20%	CD10+ (30%), TdT+	L1, L2	14q11

(TdT= terminal deoxynucleotidyl transferase)

distinctive variant of CML seen in children is called *juvenile CML*. Both the sexes are affected equally. The exact etiology is not known but an increased incidence of both AML and CML was observed years later in Japanese atomic bomb survivors implicating radiation in their etiology.

Clinical Features

The onset of CML is generally insidious. Some of the common presenting manifestations are as under:

1. Features of *anaemia* such as weakness, pallor, dyspnoea and tachycardia.
2. Symptoms due to *hypermetabolism* such as weight loss, lassitude, anorexia, night sweats.
3. *Splenomegaly* is almost always present and is frequently massive. In some patients, it may be associated with acute pain due to splenic infarction.

4. *Bleeding tendencies* such as easy bruising, epistaxis, menorrhagia and haematomas may occur.

5. Less commonly, features such as gout, visual disturbance, neurologic manifestations and priapism are present.

6. *Juvenile CML* is more often associated with lymph node enlargement than splenomegaly. Other features are frequent infections, haemorrhagic manifestations and facial rash.

Laboratory Findings

The diagnosis of CML is generally possible on blood picture alone. However, bone marrow, cytochemical stains and other investigations are of help.

I. BLOOD PICTURE. The typical blood picture in a case of CML at the time of presentation shows the

TABLE 34.5: Contrasting Features of AML and ALL.

FEATURE	AML	ALL
1. <i>Common age</i>	Adults between 15-40 years; comprise 20% of childhood leukaemias	Children under 15 years; comprise 80% of childhood leukaemias
2. <i>Physical findings</i>	Splenomegaly + Hepatomegaly + Lymphadenopathy + Bony tenderness + Gum hypertrophy +	Splenomegaly ++ Hepatomegaly ++ Lymphadenopathy ++ Bony tenderness + Meningeal involvement +
3. <i>Laboratory findings</i>	Low-to-high TLC; predominance of myeloblasts and promyelocytes in blood and bone marrow; thrombocytopenia moderate to severe.	Low-to-high TLC, predominance of lymphoblasts in blood and bone marrow; thrombocytopenia moderate to severe.
4. <i>Cytochemical stains</i>	Myeloperoxidase +, Sudan black +, NSE + in M4 and M5, acid phosphatase (diffuse) + in M4 and M5	PAS +, acid phosphatase (focal) +
5. <i>Specific therapy</i>	Cytosine arabinoside, anthracyclines (daunorubicin, adriamycin) and 6-thioguanine	Vincristine, prednisolone, anthracyclines and L-asparaginase
6. <i>Response to therapy</i>	Remission rate low, duration of remission shorter	Remission rate high, duration of remission prolonged
7. <i>Median survival</i>	12-18 months	Children without CNS prophylaxis 33 months, with CNS prophylaxis 60 months; adults 12-18 months

following features (Fig. 34.8) (COLOUR PLATE XI: CL 41):

1. Anaemia. Anaemia is usually of moderate degree and is normocytic normochromic in type. Occasional normoblasts may be present.

2. White blood cells. Characteristically, there is marked leucocytosis (approximately 200,000/ μ l or more at the time of presentation). The natural history of CML consists of 2 phases—chronic and blastic.

■ *The chronic phase of CML* begins as a myeloproliferative disorder and consists of excessive proliferation of myeloid cells of intermediate grade (i.e. myelocytes and metamyelocytes) and mature segmented neutrophils. Myeloblasts usually do not exceed 10% of cells in the peripheral blood and bone marrow. An increase in the proportion of basophils up to 10% is a characteristic feature of CML. A rising *basophilia* is indicative of impending blastic transformation. An *accelerated phase of CML* is also described in which there is progressively rising leucocytosis associated with thrombocytosis or thrombocytopenia and splenomegaly.

■ *The blastic phase or blast crisis in CML* may be myeloid or lymphoid in origin. Myeloid blast crisis in CML is more common and resembles AML. However, unlike AML, Auer rods are never seen in myeloblasts of CML in blast crisis. *Lymphoid blast crisis* in CML having the characteristics of lymphoblasts such as presence of TdT and positivity for B cell

markers (CD10, CD19) is seen in one-third cases of blastic phase in CML.

3. Platelets. The platelet count may be normal but is raised in about half the cases.

II. BONE MARROW EXAMINATION. Examination of marrow aspiration yields the following results:

1. Cellularity. Generally, there is hypercellularity with total or partial replacement of fat spaces by proliferating myeloid cells.

2. Myeloid cells. The myeloid cells predominate in the bone marrow with increased myeloid-erythroid ratio. The differential counts of myeloid cells in the marrow show similar findings as seen in the peripheral blood with predominance of myelocytes.

3. Erythropoiesis. Erythropoiesis is normoblastic but there is reduction in erythropoietic cells.

4. Megakaryocytes. Megakaryocytes are conspicuous but are usually smaller in size than normal.

5. Cytogenetics. Cytogenetic studies on blood and bone marrow cells show the characteristic chromosomal abnormality called Philadelphia (Ph) chromosome formed by reciprocal translocation between part of long arm of chromosome 22 and part of long arm of chromosome 9{(t(9;22))} seen in 70-90% cases with CML (see Fig. 34.6, page 491).

III. CYTOCHEMISTRY. The only significant finding on cytochemical stains is reduced scores of *neutrophil alkaline phosphatase (NAP)* which helps to distinguish CML from myeloid leukaemoid reaction in which case NAP scores are elevated (page 489). However, NAP scores in CML return to normal with successful therapy, corticosteroid administration and in infections.

IV. OTHER INVESTIGATIONS. A few other accompanying findings in cases of CML are:

1. Elevated serum B₁₂ and vitamin B₁₂ binding capacity.
2. Elevated serum uric acid (hyperuricaemia).

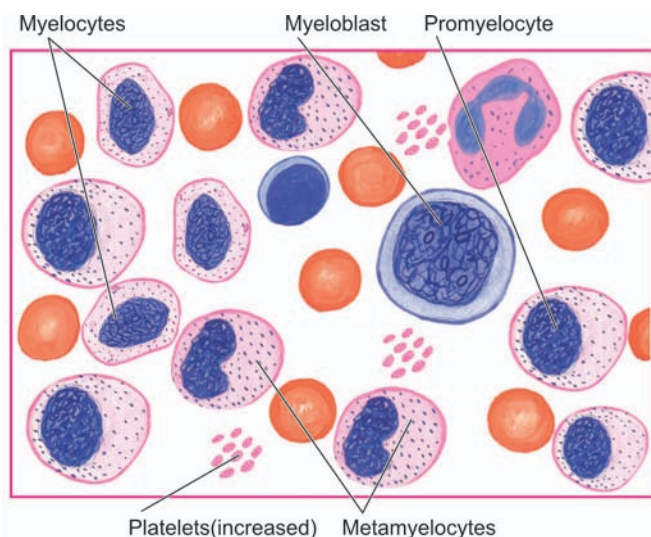


FIGURE 34.8

PBF findings in chronic myeloid leukaemia (CML).

Recently, it has been found that all cases of CML have an abnormally increased and dysregulated tyrosine kinase activity due to chimerism in *BCL-ABL* gene. This genetic abnormality has been held responsible for proliferation of leukaemic cells in CML. This has helped in targetting the defect by specific therapy in the form of synthetic inhibitor of the *BCL-ABL* kinase called STI571 that inhibits growth and proliferation of t(9;22)-bearing tumour cells.

The most common cause of death (in 80% cases) in CML is blastic transformation.

CHRONIC LYMPHOCYTIC LEUKAEMIA (CLL)

Chronic lymphocytic leukaemia constitutes about 25% of all leukaemias and is predominantly a disease of the elderly (over 50 years of age) with a male preponderance (male-female ratio 2:1). Currently, CLL is considered as part of the common lymphoid malignancy of mature B cells termed CLL/SLL i.e. CLL for cases with peripheral blood involvement while SLL for those without blood spread.

Clinical Features

The onset of disease is characteristically insidious. Common presenting manifestations are as under:

1. Features of *anaemia* such as gradually increasing weakness, fatigue and dyspnoea.
2. Enlargement of superficial *lymph nodes* is a very common finding. The lymph nodes are usually symmetrically enlarged, discrete and non-tender.
3. *Splenomegaly* and *hepatomegaly* are usual.
4. *Haemorrhagic manifestations* are found in case of CLL with thrombocytopenia.
5. *Infections*, particularly of respiratory tract, are common in CLL.
6. *Less common* are: mediastinal pressure, tonsillar enlargement, disturbed vision, and bone and joint pains.

Laboratory Findings

The diagnosis of CLL can usually be made on the basis of physical findings and blood smear examination (Fig. 34.9) (**COLOUR PLATE XI: CL 42**):

I. BLOOD PICTURE. The findings of routine blood picture are as under:

1. **Anaemia.** Anaemia is usually mild to moderate and normocytic normochromic in type. Mild reticulocytosis may be present. About 20% cases develop a Coombs'-positive autoimmune haemolytic anaemia.
2. **White blood cells.** Typically, there is marked leucocytosis but less than that seen in CML (50,000-200,000/ μ l). Usually, more than 90% of leucocytes are mature small lymphocytes. Smear cells (degenerated forms) are present. The absolute neutrophil count is, however, generally within normal range. Granulocytopenia occurs in fairly advanced disease only.
3. **Platelets.** The platelet count is normal or moderately reduced.

II. BONE MARROW EXAMINATION. The typical findings are:

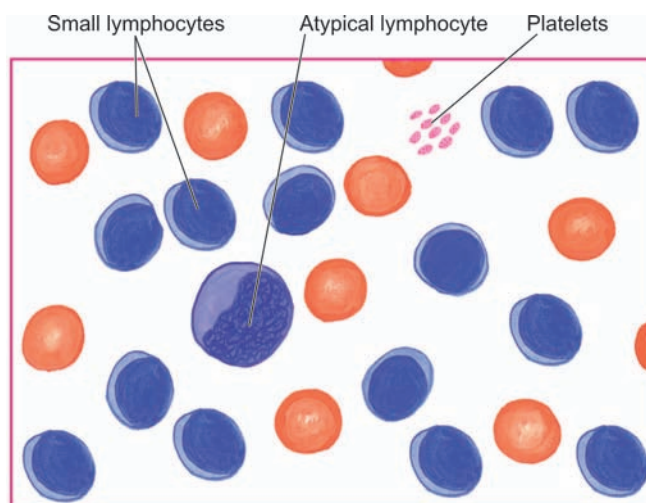


FIGURE 34.9

PBF findings in chronic lymphocytic leukaemia (CLL).

1. Increased lymphocyte count (25-95%).
2. Reduced myeloid precursors.
3. Reduced erythroid precursors.

III. OTHER INVESTIGATIONS. These are:

1. *Erythrocyte rosette test* with mouse red cells is positive in more than 95% of cases indicating that CLL is a monoclonal B cell neoplasm.
2. *Positive pan-B-cell markers* e.g. CD19, CD20, CD23, surface immunoglobulins, monoclonal light chains (λ or κ type).
3. *Serum immunoglobulin levels* are generally reduced.
4. *Coombs' test* is positive in 20% cases.

HAIRY CELL LEUKAEMIA

Hairy cell leukaemia (HCL), is an unusual and uncommon form of chronic leukaemia in which there is presence of abnormal mononuclear cells with hairy cytoplasmic projections in the bone marrow, peripheral blood and spleen. These cells are best recognised under phase contrast microscopy but may also be visible in routine blood smears. These leukaemic 'hairy cells' have characteristically positive cytochemical staining for *tartrate-resistant acid phosphatase*. The controversy on the origin of hairy cells whether these cells represent neoplastic T cells, B cells or monocytes, is settled with the molecular analysis of these cells which assigns them *B cell origin* expressing CD19, CD20 and CD22 antigen. In addition to B cell markers, hairy cells are also positive for CD11, CD25 and CD103.

Hairy cell leukaemia occurs in the older males. It is characterised clinically by the manifestations due to infiltration of reticuloendothelial organs (bone marrow, liver

and spleen) and, hence, its previous name as *leukaemic reticuloendotheliosis*. Patients have susceptibility to infection with *M. avium intercellulare*. Laboratory diagnosis is made by the presence of pancytopenia due to marrow failure and splenic sequestration, and identification of characteristic hairy cells in blood and bone marrow which are positive for tartrate-resistant acid phosphatase (TRAP).

The disease often runs a chronic course requiring supportive care. The mean survival is 4-5 years. Patients respond to splenectomy, α -interferon therapy and 2-chlorodeoxyadenosine (2-CDA).

OTHER MYELOPROLIFERATIVE DISORDERS

The myeloproliferative disorders are a group of neoplastic proliferation of multipotent haematopoietic stem cells. Besides their common stem cell origin, these disorders are closely related, occasionally leading to evolution of one entity into another during the course of the disease.

Myeloproliferative disorders include 4 disorders: chronic myeloid leukaemia (CML), polycythaemia vera, myeloid metaplasia with myelosclerosis, and essential thrombocythosis (or primary/idiopathic thrombocythaemia). The CML has already been discussed; other disorders are considered here.

POLYCYTHAEMIA VERA

DEFINITION AND ETIOLOGY. Polycythaemia vera (PV) is characterised by increased production of all myeloid elements resulting in pancytosis, elevated haemoglobin concentration and splenomegaly. The term 'polycythaemia vera' or 'polycythaemia rubra vera' is used for *primary or idiopathic polycythaemia* only. *Secondary polycythaemia or erythrocytosis* may occur due to several causes e.g. high altitude, cardiovascular disease, pulmonary disease with alveolar hypoventilation, heavy smoking, inappropriate increase in erythropoietin (renal cell carcinoma, hydronephrosis, hepatocellular carcinoma, cerebellar haemangioblastoma, massive uterine leiomyoma); sometimes relative or spurious polycythaemia may result from plasma loss such as in burns and in dehydration from vomiting or water deprivation. None of the secondary causes of polycythaemia are associated with splenic enlargement or increased leucocytes and platelets which are typical of PV.

In PV, unlike secondary polycythaemia, the *erythropoietin levels in serum and urine are reduced*. Thus, proliferation of haematopoietic cells in the bone marrow in PV is independent of the usual erythropoietin regulatory mechanism.

CLINICAL FEATURES. PV is a disease of late middle life and is slightly more common in males. The disease generally runs a chronic but slowly progressive course. Clinical features are the result of hyperviscosity, hypervolaemia, hypermetabolism and decreased cerebral perfusion. These are:

1. Headache, vertigo, tinnitus, visual alterations syncope or even coma.
2. Increased risk of thrombosis due to accelerated atherosclerosis.
3. Increased risk of haemorrhages due to increased blood volume and intrinsic platelet dysfunction e.g. epistaxis, peptic ulcer disease.
4. Splenomegaly producing abdominal fullness.
5. Pruritus, especially after a bath.
6. Increased risk of urate stones and gout due to hyperuricaemia.

LABORATORY FINDINGS. PV is diagnosed by the following haematologic findings:

1. Raised haemoglobin concentration (above 17.5 g/dl in males and 15.5 g/dl in females).
2. Erythrocytosis (above 6 million/ μ l in males and 5.5 million/ μ l in females).
3. Haematocrit (PCV) above 55% in males and above 47% in females.
4. Mild to moderate leucocytosis (15,000-25,000/ μ l) with basophilia and raised neutrophil alkaline phosphatase scores.
5. Thrombocytosis with defective platelet function.
6. Bone marrow examination reveals erythroid hyperplasia or panhyperplasia.
7. Cytogenetic abnormalities such as trisomy are found in 10% cases of PV.

TREATMENT. Therapy is aimed at maintaining normal blood counts. It includes *phlebotomy* (venesection) by blood letting; *cytotoxic myelosuppression* with busulfan, chlorambucil and cyclophosphamide; and in severe cases *intravenous administration of radioactive phosphorus* (^{32}P). Patients receiving phlebotomy alone may survive for 10-12 years. About 25% patients progress to myelofibrosis. A small proportion of patients develop secondary haematologic malignancies such as AML, non-Hodgkin's lymphoma and multiple myeloma. The majority of patients, however, die of vascular complications.

MYELOID METAPLASIA WITH MYELOFIBROSIS

DEFINITION AND ETIOLOGY. Myeloid metaplasia with myelofibrosis, also called agnogenic (of unknown

origin) myeloid metaplasia, primary myelofibrosis and myelosclerosis, is characterised by proliferation of neoplastic stem cells at multiple sites outside the bone marrow (i.e. extramedullary haematopoiesis), especially in the liver and spleen. About 25% cases have a preceding history of polycythaemia. Secondary myelofibrosis, on the other hand, develops in association with certain well-defined marrow disorders, or are the result of toxic action of chemical agents or irradiation.

CLINICAL FEATURES. The disease begins in the late middle life and is gradual in onset. Both sexes are affected equally. The symptomatology includes the following:

1. Anaemia with constitutional symptoms such as fatigue, weakness and anorexia.
2. Massive splenomegaly producing abdominal discomfort, pain and dyspnoea.
3. Hepatomegaly is present in half the cases.
4. Petechial and other bleeding problems are found in about 20% cases.
5. Less common findings are lymphadenopathy, jaundice, ascites, bone pain and hyperuricaemia.

LABORATORY FINDINGS. These are as under:

1. *Mild anaemia* is usual except in cases where features of polycythaemia vera are coexistent.
2. *Leucocytosis* at the time of presentation but later there may be leucopenia.
3. *Thrombocytosis* initially but advanced cases show thrombocytopenia.
4. *Peripheral blood smear* shows bizarre red cell shapes, tear drop poikilocytes, basophilic stippling, nucleated red cells, immature leucocytes (i.e. leucoerythroblastic reaction), basophilia and giant platelet forms.
5. *Bone marrow aspiration* is generally unsuccessful and yields 'dry tap'. Examination of trephine biopsy shows focal areas of hypercellularity and increased reticulin network and variable amount of collagen in which clusters of megakaryocytes are seen well preserved.

6. *Extramedullary haematopoiesis* can be documented by liver biopsy or splenic aspiration.

ESSENTIAL THROMBOCYTOSIS

DEFINITION AND ETIOLOGY. Essential thrombocytosis or primary (idiopathic) thrombocythaemia is another myeloproliferative disorder characterised by markedly elevated platelet count in the absence of any recognisable stimulus. *Secondary or reactive thrombocytosis*, on the other hand, occurs in response to known stimuli such as: chronic infection, haemorrhage, post-operative state, chronic iron deficiency, malignancy, rheumatoid arthritis and postsplenectomy. Essential thrombocytosis represents an overproduction of platelets from megakaryocyte colonies without any added stimulus. Though an elevated platelet count is the dominant feature, other cell lines are also involved in the expansion of neoplastic clone.

CLINICAL FEATURES. The condition has an insidious onset and is more frequent in older people. Haemorrhagic and thrombotic events are common. These include:

1. Arterial or venous thrombosis.
2. Easy bruisability following minor trauma.
3. Spontaneous bleeding.
4. Transient ischaemic attack or frank stroke due to platelet aggregation in microvasculature of the CNS.

LABORATORY FINDINGS. The prominent laboratory features pertain to platelets. These include the following:

1. Sustained elevation in platelet count (above 400,000 μl).
2. Blood film shows many large platelets, megakaryocyte fragments and hypogranular forms.
3. Consistently abnormal platelet functions, especially abnormality in platelet aggregation.
4. Bone marrow examination reveals large number of hyperdiploid megakaryocytes and variable amount of increased fibrosis.



Platelets and Haemorrhagic Diathesis

THROMBOPOIESIS

Platelets are formed in the bone marrow by a process of fragmentation of the cytoplasm of megakaryocytes. Platelet production appears to be under the control of thrombopoietin, the nature and origin of which are not yet established. The stages in platelet production are: megakaryoblast, promegakaryocyte, megakaryocyte, and discoid platelets (Fig. 35.1).

MEGAKARYOBLAST. The earliest precursor of platelets in the bone marrow is megakaryoblast. It arises from haematopoietic stem cell by a process of differentiation.

PROMEGAKARYOCYTE. A megakaryoblast undergoes endo-reduplication of nuclear chromatin i.e. nuclear chromatin replicates repeatedly in multiples of two without division of the cell. Ultimately, a large cell containing up to 32 times the normal diploid content of nuclear DNA (polyploidy) is formed when further nuclear replication ceases and cytoplasm becomes granular.

MEGAKARYOCYTE. A mature megakaryocyte is a large cell, 30-90 μm in diameter, and contains 4-16 nuclear lobes having coarsely clumped chromatin. The cytoplasm is abundant, light blue in colour and contains

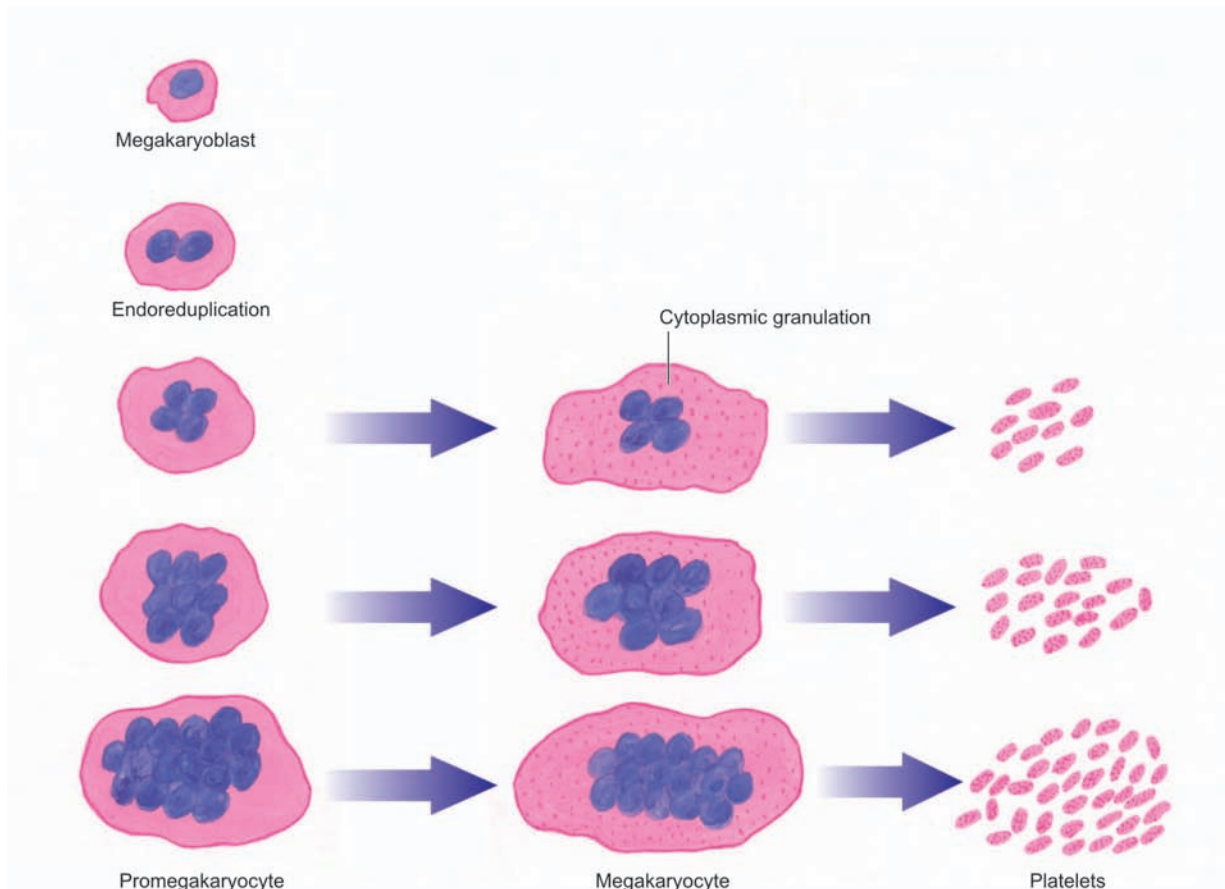


FIGURE 35.1

Thrombopoiesis.

red-purple granules. Platelets are formed from pseudopods of megakaryocyte cytoplasm which get detached into the blood stream. Each megakaryocyte may form up to 4000 platelets. The formation of platelets from the stem cell takes about 10 days.

PLATELETS. Platelets are small (1-4 μm in diameter), discoid, non-nucleate structures containing red-purple granules. The normal platelet count ranges from 150,000-400,000/ μl and its life-span is 7-10 days. Newly-formed platelets spend 24-36 hours in the spleen before being released into circulation but splenic stasis does not cause any injury to the platelets normally.

The main **function** of platelets is in haemostasis which includes two closely linked processes:

1. Primary haemostasis is the term used for platelet plug formation at the site of injury. It is an immediate phenomenon appearing within seconds of injury and is responsible for cessation of bleeding from microvasculature. Primary haemostasis involves three steps: platelet adhesion, platelet granule release and platelet aggregation which are regulated by changes in membrane phospholipids, and calcium (Fig. 35.2). At molecular level, these important events are depicted diagrammatically in Fig. 35.3 and briefly outlined below:

■ **Platelet adhesion:** Platelets adhere to collagen in the subendothelium due to presence of receptor on platelet surface, glycoprotein (Gp) Ia-IIa which is an integrin. The adhesion to the vessel wall is further stabilised by von Willebrand factor, an adhesion glycoprotein. This is achieved by formation of a link between von Willebrand factor and another platelet receptor, GpIb-IX complex.

■ **Platelet release:** The adherent platelets release preformed granules that includes ADP, factor Va, thrombospondin, platelet-derived growth factor (PDGF), von Willebrand factor (vWF), fibronectin, fibrinogen, heparinase and thromboxane A2 (Fig. 35.2).

■ **Platelet aggregation:** This process is mediated by fibrinogen which forms bridge between adjacent platelets via glycoprotein receptors on platelets, GpIIb-IIIa.

2. Secondary haemostasis involves plasma coagulation system resulting in fibrin plug formation and takes several minutes for completion. This is discussed in detail in Chapter 17.

HAEMORRHAGIC DIATHESIS (BLEEDING DISORDERS)

Bleeding disorders or haemorrhagic diatheses are a group of disorders characterised by defective haemostasis with abnormal bleeding. The tendency to bleeding

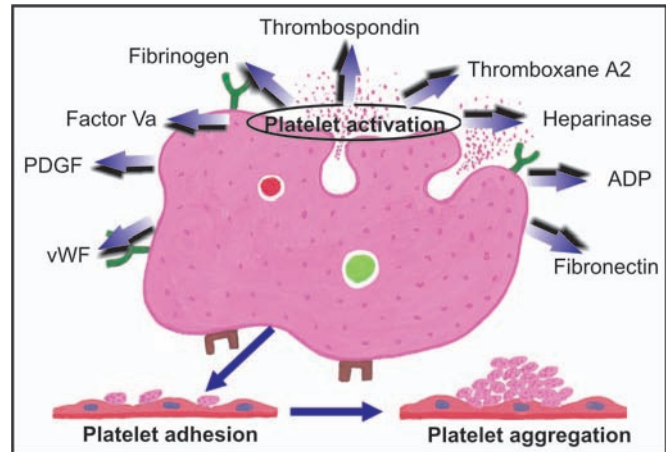


FIGURE 35.2

Main events in primary haemostasis—platelet adhesion, release (activation) and aggregation.

may be *spontaneous* in the form of small haemorrhages into the skin and mucous membranes (e.g. petechiae, purpura, ecchymoses), or there may be excessive external or internal bleeding *following trivial trauma* and surgical procedure (e.g. haematoma, haemarthrosis etc).

The causes of haemorrhagic diatheses may or may not be related to platelet abnormalities. These causes are broadly divided into the following 4 groups:

- I. Haemorrhagic diathesis due to vascular abnormalities.
- II. Haemorrhagic diathesis related to platelet abnormalities.
- III. Disorders of coagulation factors.
- IV. Combination of all these as occurs in disseminated intravascular coagulation (DIC).

Before discussing the bleeding disorders, it is considered desirable to describe briefly the broad outlines of scheme of investigations to be carried out in such a case.

INVESTIGATIONS OF HAEMOSTATIC FUNCTION

Normal haemostatic mechanism and thrombogenesis were discussed in Chapter 17. In general, the haemostatic mechanisms have 2 primary functions:

- To promote *local haemostasis* at the site of injured blood vessel.
- To ensure that the circulating blood remains in *fluid state* while in the vascular bed i.e. to prevent the occurrence of generalised thrombosis.

In order to perform these haemostatic functions, a delicate balance must exist among at least 5 components

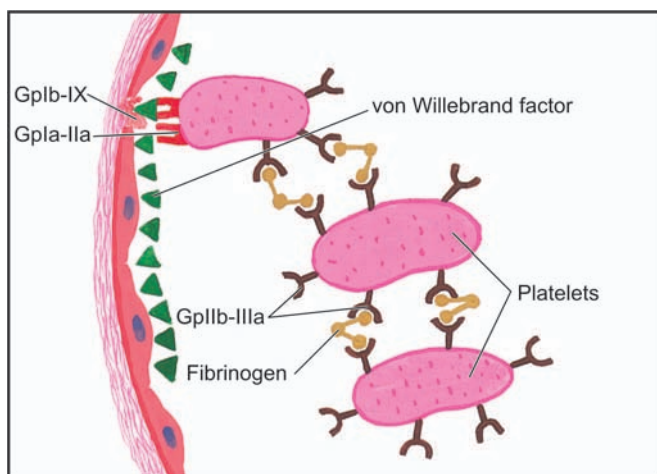


FIGURE 35.3

Molecular mechanisms in platelet adhesion and release reaction (Gp=glycoprotein).

(Fig. 35.4): (i) blood vessel wall; (ii) platelets; (iii) plasma coagulation factors; (iv) inhibitors; and (v) fibrinolytic system.

Anything that interferes with any of these components results in defective haemostasis with abnormal bleeding. In order to establish a definite diagnosis in any case suspected to have abnormal haemostatic functions, the following scheme is followed:

A. Comprehensive *clinical evaluation*, including the patient's history, family history and details of the site, frequency and character of haemostatic defect.

B. Series of *screening tests* for assessing the abnormalities in various components involved in maintaining haemostatic balance.

C. *Specific tests* to pinpoint the cause.

A brief review of general principles of tests used to investigate haemostatic abnormalities is presented below and summarised in Table 35.1.

A. Investigations of Disordered Vascular Haemostasis

Disorders of vascular haemostasis may be due to increased vascular permeability, reduced capillary strength and failure to contract after injury. Tests of defective vascular function are as under:

1. BLEEDING TIME. This simple test is based on the principle of formation of haemostatic plug following a standard incision on the volar surface of the forearm and the time the incision takes to stop bleeding is measured. The test is dependent upon capillary function as well as on platelet number and ability of platelets to adhere to form aggregates. Normal range is 3-8 minutes. A prolonged bleeding time may be due to:

- i) Thrombocytopenia.
- ii) Disorders of platelet function.
- iii) von Willebrand's disease.
- iv) Vascular abnormalities (e.g. in Ehlers-Danlos syndrome).
- v) Severe deficiency of factor V and XI.

TABLE 35.1: Screening Tests for Haemostasis (Coagulation Tests).

LABORATORY TEST	FACTOR/FUNCTION MEASURED	ASSOCIATED DISORDERS
1. <i>Bleeding time</i>	Platelet function, vascular integrity	i) Qualitative disorders of platelets ii) von Willebrand's disease iii) Quantitative disorders of platelets iv) Acquired vascular disorders
2. <i>Platelet count</i>	Quantification of platelets	i) Thrombocytopenia ii) Thrombocytosis
3. <i>Prothrombin time</i>	Evaluation of extrinsic and common pathway (deficiency of factors I, II, V, VII, X)	i) Oral anticoagulant therapy ii) DIC iii) Liver disease
4. <i>Partial thromboplastin time</i>	Evaluation of intrinsic and common pathway (deficiency of factors I, II, V, VIII, IX, X, XI, XII)	i) Parenteral heparin therapy ii) DIC iii) Liver disease
5. <i>Thrombin time</i>	Evaluation of common pathway	i) Afibrinogenaemia ii) DIC iii) Parenteral heparin therapy

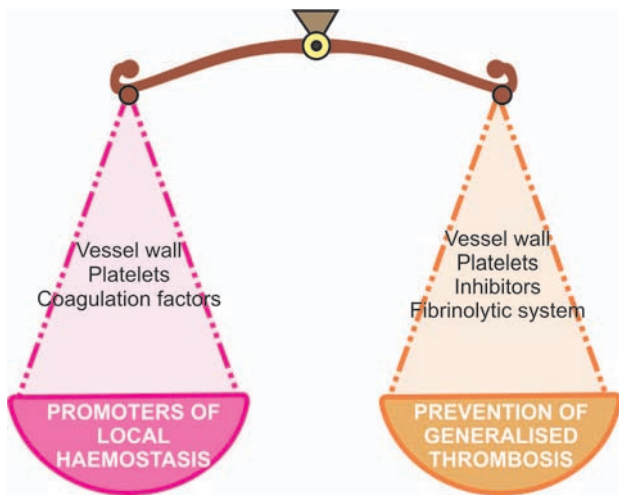


FIGURE 35.4

The Haemostatic balance.

2. HESS CAPILLARY RESISTANCE TEST (TOURNIQUET TEST). This test is done by tying sphygmomanometer cuff to the upper arm and raising the pressure in it between diastolic and systolic for 5 minutes. After deflation, the number of petechiae appearing in the next 5 minutes in 3 cm² area over the cubital fossa are counted. Presence of more than 20 petechiae is considered a positive test. The test is positive in increased capillary fragility as well as in thrombocytopenia.

B. Investigation of Platelets and Platelet Function

Haemostatic disorders are most commonly due to abnormalities in platelet number, morphology or function.

I. SCREENING TESTS. The screening tests carried out for assessing these are:

1. Peripheral blood platelet count.
2. Skin bleeding time.
3. Examination of fresh blood film to see the morphologic abnormalities of platelets.

II. SPECIAL TESTS. If these screening tests suggest a disorder of platelet function, the following platelet function tests may be carried out:

1. *Platelet adhesion tests* such as retention in a glass bead column, and other sophisticated techniques.
2. *Aggregation tests* which are turbidometric techniques using ADP, collagen or ristocetin.
3. *Granular content* of the platelets and their release can be assessed by electron microscopy or by measuring the substances released.

4. *Platelet coagulant activity* is measured indirectly by prothrombin consumption index.

C. Investigation of Blood Coagulation

The normal blood coagulation system consisting of intrinsic and extrinsic pathways and the common pathway is shown in Fig. 35.5.

I. SCREENING TESTS. Tests for blood coagulation system include a battery of screening tests. These are as under:

1. Whole blood coagulation time. The estimation of whole blood coagulation time done by various capillary and tube methods is of limited value since it is an insensitive and nonspecific test. Normal range is 4-9 minutes at 37°C.

2. Activated partial thromboplastin time (APTT) or partial thromboplastin time with kaolin (PTTK). This test is used to measure the intrinsic system factors (VIII, IX, XI and XII) as well as factors common to both intrinsic and extrinsic systems (factors X, V, prothrombin and fibrinogen). The test consists of addition of 3 substances to the plasma—calcium, phospholipid and a surface activator such as kaolin. The normal range is 30-40 seconds. The common causes of a prolonged PTTK (or APTT) are:

- i) Parenteral administration of heparin.
- ii) Disseminated intravascular coagulation.
- iii) Liver disease.
- iv) Circulating anticoagulants.

3. One-stage prothrombin time (PT). PT measures the extrinsic system factor VII as well as factors in the common pathway. In this test, tissue thromboplastin (e.g. brain extract) and calcium are added to the test. The normal PT in this test is 10-14 seconds. The common causes of prolonged one-stage PT are:

- i) Administration of oral anticoagulant drugs.
- ii) Liver disease, especially obstructive liver disease.
- iii) Vitamin K deficiency.
- iv) Disseminated intravascular coagulation.

4. Measurement of fibrinogen. The *screening tests* for fibrinogen deficiency are semiquantitative fibrinogen titre and thrombin time (TT). The normal value of thrombin time is under 20 seconds, while a fibrinogen titre in plasma dilution up to 32 is considered normal. The common causes for higher values in both these tests are:

- i) Hypofibrinogenaemia (e.g. in DIC).
- ii) Raised concentration of FDP.
- iii) Presence of heparin.

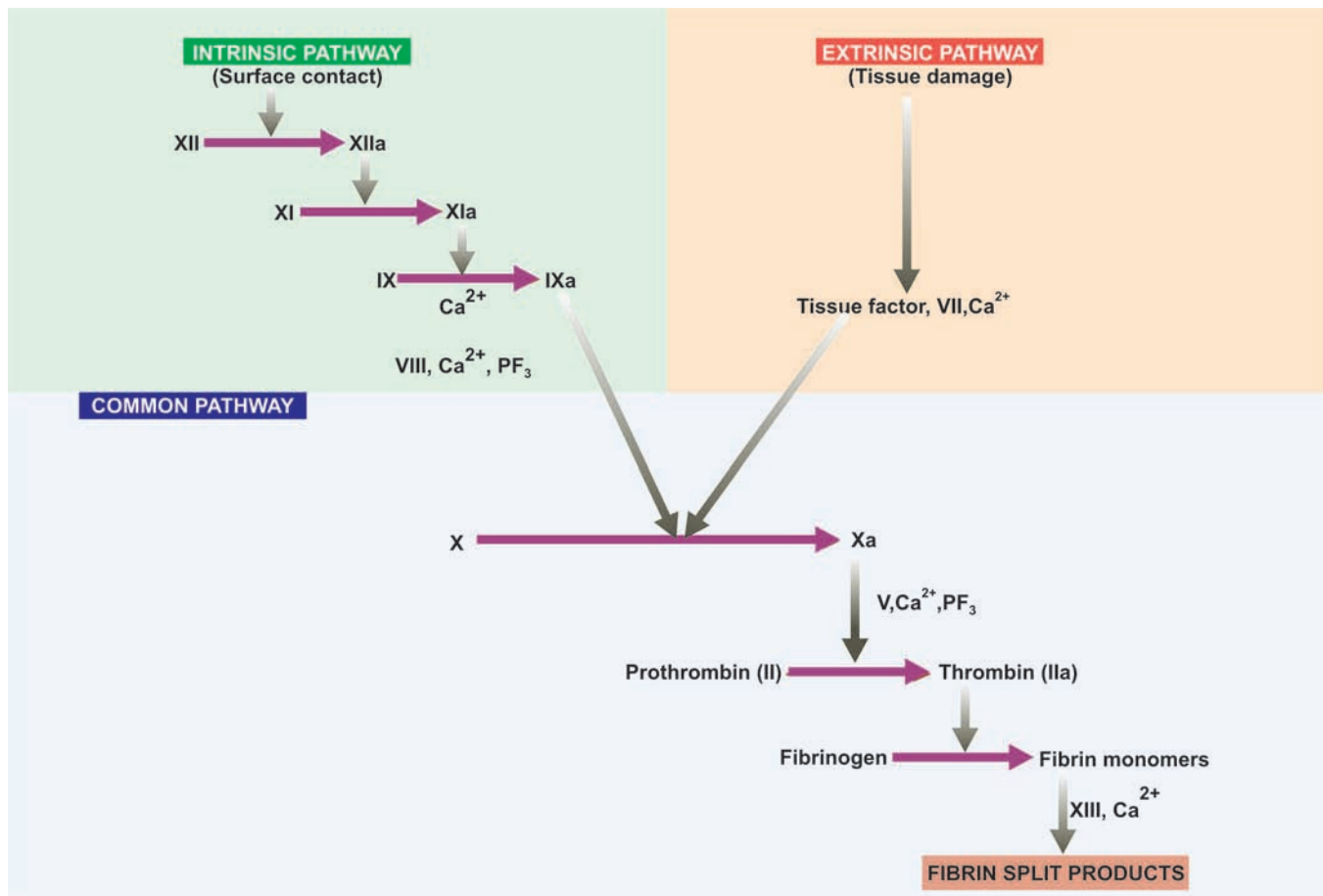


FIGURE 35.5

Pathways of blood coagulation.

II. SPECIAL TESTS. In the presence of an abnormality in screening tests, detailed investigations for the possible cause are carried out. These include the following:

1. Coagulation factor assays. These bioassays are based on results of PTTK or PT tests and employ the use of substrate plasma that contains all other coagulation factors except the one to be measured. The unknown level of the factor activity is compared with a standard control plasma with a known level of activity. Results are expressed as percentage of normal activity.

2. Quantitative assays. The coagulation factors can be quantitatively assayed by immunological and other chemical methods.

D. Investigation of Fibrinolytic System

Increased levels of circulating plasminogen activator are present in patients with hyperfibrinolysis. *Screening tests*

done to assess these abnormalities in fibrinolytic system are:

1. Estimation of fibrinogen.
2. Fibrin degradation products (FDP) in the serum.
3. Ethanol gelation test.
4. Euglobin or whole blood lysis time.

More *specific tests* include: functional assays, immunological assays by ELISA, and chromogenic assays of plasminogen activators, plasminogen, plasminogen activator inhibitor, and FDP.

HAEMORRHAGIC DIATHESSES DUE TO VASCULAR DISORDERS

Vascular bleeding disorders, also called non-thrombocytopenic purpuras or vascular purpuras, are normally mild and characterised by petechiae, purpuras or ecchymoses confined to the skin and mucous membranes. The pathogenesis of bleeding is poorly under-

stood since majority of the standard screening tests of haemostasis including the bleeding time, coagulation time, platelet count and platelet function, are usually normal. Vascular purpuras arise from damage to the capillary endothelium, abnormalities in the subendothelial matrix or extravascular connective tissue that supports the blood vessels, or from formation of abnormal blood vessels.

Vascular bleeding disorders may be *inherited* or *acquired*.

A. Inherited Vascular Bleeding Disorders

A few examples of hereditary vascular disorders are given below:

1. Hereditary haemorrhagic telangiectasia (Osler-Weber-Rendu disease). This is an uncommon inherited autosomal dominant disorder. The condition begins in childhood and is characterised by abnormally telangiectatic (dilated) capillaries. These telangiectasias develop particularly in the skin, mucous membranes and internal organs and are the source of frequent episodes of bleeding from nose and gastrointestinal tract.

2. Inherited disorders of connective tissue matrix. These include Marfan's syndrome, Ehlers-Danlos syndrome and pseudoxanthoma elasticum, all of which have inherited defect in the connective tissue matrix and, thus, have fragile skin vessels and easy bruising.

B. Acquired Vascular Bleeding Disorders

Several acquired conditions are associated with vascular purpuras. These are as under:

1. Henoch-Schönlein purpura. Henoch-Schönlein or anaphylactoid purpura is a self-limited type of hypersensitivity vasculitis occurring in children and young adults. Circulating immune complexes are deposited in the vessel wall consisting of IgA, C3 and fibrin, and in some cases, properdin suggesting activation of alternate complement pathway as the trigger event. The hypersensitivity vasculitis produces purpuric rash on the extensor surfaces of arms, legs and on the buttocks, as well as haematuria, colicky abdominal pain due to bleeding into the GIT, polyarthralgia and acute nephritis. In spite of these haemorrhagic features, all coagulation tests are normal.

2. Haemolytic-uraemic syndrome. Haemolytic-uraemic syndrome is a disease of infancy and early childhood in which there is bleeding tendency and varying degree of acute renal failure. The disorder remains confined to the kidney where hyaline thrombi are seen in the glomerular capillaries.

3. Simple easy bruising (Devil's pinches). Easy bruising of unknown cause is a common phenomenon in women of child-bearing age group.

4. Infection. Many infections cause vascular haemorrhages either by causing toxic damage to the endothelium or by DIC. These are especially prone to occur in septicaemia and severe measles.

5. Drug reactions. Certain drugs form antibodies and produce hypersensitivity (or leucocytoclastic) vasculitis responsible for abnormal bleeding.

6. Steroid purpura. Long-term steroid therapy or Cushing's syndrome may be associated with vascular purpura due to defective vascular support.

7. Senile purpura. Atrophy of the supportive tissue of cutaneous blood vessels in old age may cause senile atrophy, especially in the dorsum of forearm and hand.

8. Scurvy. Deficiency of vitamin C causes defective collagen synthesis which causes skin bleeding as well as bleeding into muscles, and occasionally into the gastrointestinal and genitourinary tracts.

HAEMORRHAGIC DIATHESSES DUE TO PLATELET DISORDERS

Disorders of platelets produce bleeding disorders by one of the following 2 mechanisms:

A. *Due to reduction in the number of platelets* i.e. various forms of thrombocytopenias.

B. *Due to defective platelet functions.*

A. THROMBOCYTOPENIAS

Thrombocytopenia is defined as a reduction in the peripheral blood platelet count below the lower limit of normal i.e. below 150,000/ μ l. Thrombocytopenia is associated with abnormal bleeding that includes spontaneous skin purpura and mucosal haemorrhages as well as prolonged bleeding after trauma. However, spontaneous haemorrhagic tendency becomes clinically evident only after severe depletion of the platelet count to level of about 20,000/ μ l.

Thrombocytopenia may result from 4 main groups of causes:

1. Impaired platelet production.
2. Accelerated platelet destruction.
3. Splenic sequestration.
4. Dilutional loss.

A list of causes of thrombocytopenia is given in Table 35.2. Three of the common and important causes—drug-induced thrombocytopenia, idiopathic thrombo-

TABLE 35.2: Causes of Thrombocytopenia.

I. IMPAIRED PLATELET PRODUCTION1. *Generalised bone marrow failure e.g.*

Aplastic anaemia, leukaemia, myelofibrosis, megaloblastic anaemia, marrow infiltrations (carcinomas, lymphomas, multiple myeloma, storage diseases).

2. *Selective suppression of platelet production e.g.*

Drugs (quinine, quinidine, sulfonamides, PAS, rifampicin, anticancer drugs, thiazide diuretics), alcohol intake.

II. ACCELERATED PLATELET DESTRUCTION1. *Immunologic thrombocytopenias e.g.*

ITP (acute and chronic), neonatal and post-transfusion (isoimmune), drug-induced, secondary immune 'thrombocytopenia (post-infection, SLE, AIDS, CLL, lymphoma).

2. *Increased consumption e.g.*

DIC, TTP, giant haemangiomas, microangiopathic haemolytic anaemia.

III. SPLENIC SEQUESTRATION

Splenomegaly

IV. DILUTIONAL LOSS

Massive transfusion of old stored blood to bleeding patients.

cytopenic purpura (ITP), and thrombotic thrombocytopenic purpura (TTP), are discussed below.

Drug-induced Thrombocytopenia

Many commonly used drugs cause thrombocytopenia by depressing megakaryocyte production. In most cases, an immune mechanism by formation of drug-antibody complexes is implicated in which the platelet is damaged as an 'innocent bystander'. Drug-induced thrombocytopenia is associated with many commonly used drugs and includes: chemotherapeutic agents (alkylating agents, anthracyclines, antimetabolites), certain antibiotics (sulfonamides, PAS, rifampicin, penicillins), drugs used in cardiovascular diseases (digitoxin, thiazide diuretics), heparin and excessive consumption of ethanol.

Clinically, the patient presents with acute purpura. The platelet count is markedly lowered, often below 10,000/ μ l and the bone marrow shows normal or increased number of megakaryocytes. The immediate treatment is to stop or replace the suspected drug with instruction to the patient to avoid taking the offending drug in future. Occasional patients may require temporary support with glucocorticoids, plasmapheresis or platelet transfusions.

Idiopathic Thrombocytopenic Purpura (ITP)

Idiopathic thrombocytopenic purpura (ITP), also called immunologic thrombocytopenic purpura, is characterised by immunologic destruction of platelets and normal or increased megakaryocytes in the bone marrow.

PATHOGENESIS. On the basis of duration of illness, ITP is classified into acute and chronic forms, both of which have different pathogenesis.

Acute ITP. This is a self-limited disorder, seen most frequently in children following recovery from a viral illness (e.g. viral hepatitis, infectious mononucleosis, CMV infection) or an upper respiratory illness. The onset of acute ITP is sudden and thrombocytopenia is severe but recovery occurs within a few weeks to 6 months. The mechanism of acute ITP is by formation of *immune complexes* containing viral antigens, and by formation of *antibodies* against viral antigens which crossreact with platelets and lead to their immunologic destruction.

Chronic ITP. Chronic ITP occurs more commonly in adults, particularly in women of child-bearing age (20-40 years). The disorder develops insidiously and persists for several years. Though chronic ITP is idiopathic, similar immunologic thrombocytopenia may be seen in association with SLE, AIDS and autoimmune thyroiditis. The pathogenesis of chronic ITP is explained by formation of *anti-platelet autoantibodies*, usually by platelet-associated IgG humoral antibodies synthesised mainly in the spleen. These antibodies are directed against target antigens on the platelet glycoproteins, Gp IIb-IIIa and Gp Ib-IX complex. Some of the antibodies directed against platelet surface also interfere in their function. The mechanism of platelet destruction is similar to that seen in autoimmune haemolytic anaemias. Sensitised platelets are destroyed mainly in the spleen and rendered susceptible to phagocytosis by cells of the reticuloendothelial system.

CLINICAL FEATURES. The clinical manifestation of ITP may develop abruptly as in cases of acute ITP, or the onset may be insidious as occurs in majority of cases of chronic ITP. The usual manifestations are petechial haemorrhages, easy bruising, and mucosal bleeding such as menorrhagia in women, nasal bleeding, bleeding from gums, melaena and haematuria. Intracranial haemorrhage is, however, rare. Splenomegaly and hepatomegaly may occur in cases with chronic ITP but lymphadenopathy is quite uncommon in either type of ITP.

LABORATORY FINDINGS. The diagnosis of ITP can be suspected on clinical features after excluding the known causes of thrombocytopenia and is supported by the following haematologic findings (Fig. 35.6):

1. *Platelet count* is markedly reduced, usually in the range of 10,000-50,000/ μ L.
2. *Blood film* shows only occasional platelets which are often large in size.
3. *Bone marrow* shows increased number of megakaryocytes which have large non-lobulated single nuclei and may have reduced cytoplasmic granularity and presence of vacuoles.
4. With sensitive techniques, *anti-platelet IgG antibody* can be demonstrated on platelet surface or in the serum of patients.
5. *Platelet survival studies* reveal markedly reduced platelet life-span, sometimes less than one hour as against normal life-span of 7-10 days.

Treatment

Spontaneous recovery occurs in 90% cases of acute ITP, while only less than 10% cases of chronic ITP recover

spontaneously. Treatment is directed at reducing the level and source of autoantibodies and reducing the rate of destruction of sensitised platelets. This is possible by corticosteroid therapy, immunosuppressive drugs (e.g. vincristine, cyclophosphamide and azathioprine) and splenectomy. The beneficial effects of splenectomy in chronic ITP are due to both removal of the major site of platelet destruction and the major source of auto-antibody synthesis. Platelet transfusions are helpful as a palliative measure only in patients with severe haemorrhage.

Thrombotic Thrombocytopenic Purpura (TTP)

Thrombotic thrombocytopenic purpura (TTP) is an uncommon but often fulminant and lethal disorder occurring in young adults. It is essentially characterised by triad of *thrombocytopenia, microangiopathic haemolytic anaemia and formation of hyaline fibrin microthrombi* within the microvasculature throughout the body. The intra-vascular microthrombi are composed predominantly of platelets and fibrin. The widespread presence of these platelet microthrombi is responsible for thrombocytopenia due to increased consumption of platelets, microangiopathic haemolytic anaemia and protean clinical

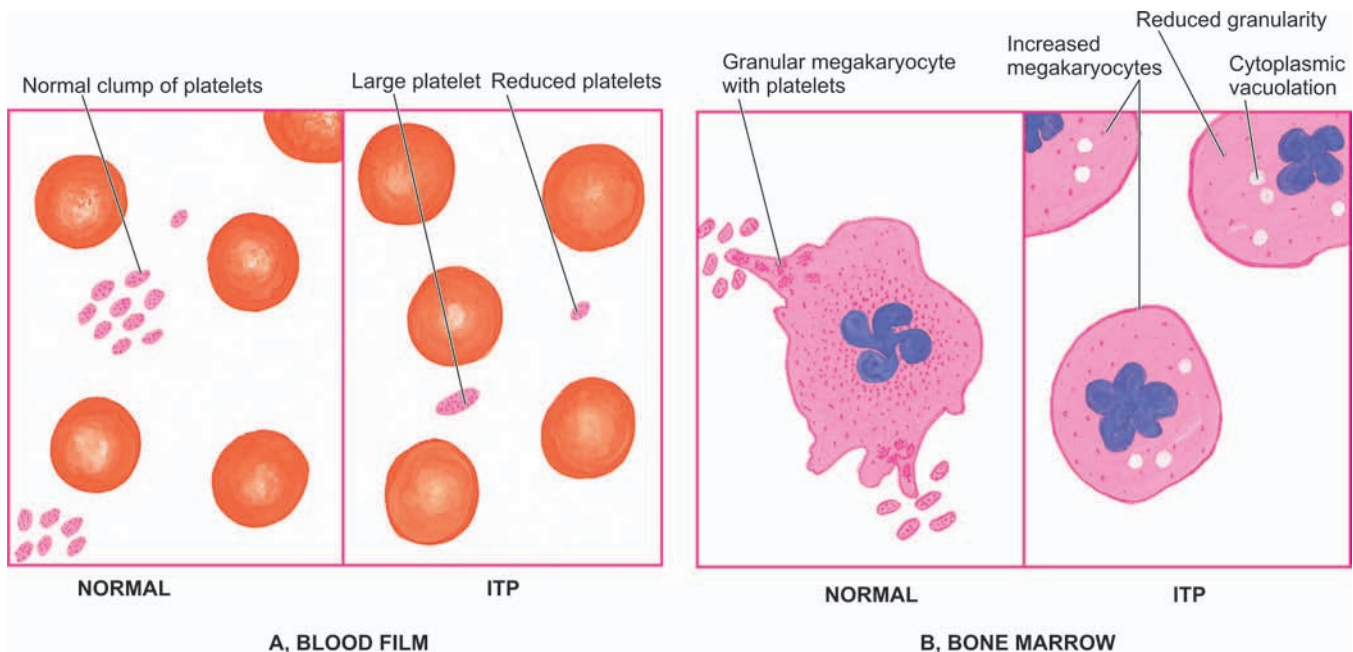


FIGURE 35.6

Laboratory findings of ITP contrasted with those found in a normal individual. A, Peripheral blood in ITP shows presence of reduced number of platelets which are often large. B, Bone marrow in ITP shows characteristically increased number of megakaryocytes with single non-lobulated nuclei and reduced cytoplasmic granularity and presence of vacuoles. The other cells in the peripheral blood and marrow are normal and are not included in the pictures here.

manifestations involving different organs and tissues throughout the body.

PATHOGENESIS. Unlike DIC, a clinicopathologically related condition, activation of the clotting system is not the primary event in formation of microthrombi. TTP is initiated by endothelial injury followed by release of von Willebrand factor and other procoagulant material from endothelial cells, leading to the formation of microthrombi. The trigger for the endothelial injury comes from immunologic damage by diverse conditions such as in pregnancy, metastatic cancer, high-dose chemotherapy, HIV infection, and mitomycin C.

CLINICAL FEATURES. The clinical manifestations of TTP are due to microthrombi in the arterioles, capillaries and venules throughout the body. Besides features of thrombocytopenia and microangiopathic haemolytic anaemia, characteristic findings include fever, transient neurologic deficits and renal failure. The spleen may be palpable.

LABORATORY FINDINGS. The diagnosis can be made from the following findings:

1. Thrombocytopenia.
2. Microangiopathic haemolytic anaemia with negative Coombs' test.
3. Leucocytosis, sometimes with leukaemoid reaction.
4. Bone marrow examination reveals normal or slightly increased megakaryocytes accompanied with some myeloid hyperplasia.
5. Diagnosis is, however, established by examination of biopsy (e.g. from gingiva) which demonstrates typical microthrombi in arterioles, capillaries and venules, unassociated with any inflammatory changes in the vessel wall.

B. DISORDERS OF PLATELET FUNCTIONS

Defective platelet function is suspected in patients who show skin and mucosal haemorrhages and have prolonged bleeding time but a normal platelet count. These disorders may be hereditary or acquired.

1. Hereditary Disorders

Depending upon the predominant functional abnormality, inherited disorders of platelet functions are classified into the following 3 groups:

1. **DEFECTIVE PLATELET ADHESION.** These are as under:
 - i) *Bernard-Soulier syndrome* is an autosomal recessive disorder with inherited deficiency of a platelet

membrane glycoprotein which is essential for adhesion of platelets to vessel wall.

- ii) *In von Willebrand's disease*, there is defective platelet adhesion as well as deficiency of factor VIII (page 510).

2. **DEFECTIVE PLATELET AGGREGATION.** In *thrombasthenia (Glanzmann's disease)*, there is failure of primary platelet aggregation with ADP or collagen due to inherited deficiency of two of platelet membrane glycoproteins.

3. **DISORDERS OF PLATELET RELEASE REACTION.** These disorders are characterised by normal initial aggregation of platelets with ADP or collagen but the subsequent release of ADP, prostaglandins and 5-HT is defective due to complex intrinsic deficiencies.

2. Acquired Disorders

Acquired defects of platelet functions include the following clinically significant examples:

1. **ASPIRIN THERAPY.** Prolonged use of aspirin leads to easy bruising and abnormal bleeding time. This is because aspirin inhibits the enzyme cyclooxygenase, and thereby suppresses the synthesis of prostaglandins which are involved in platelet aggregation as well as release reaction. The anti-platelet effect of aspirin is clinically made use of in preventing major thromboembolic disease in recurrent myocardial infarction.

2. **OTHERS.** Several other acquired disorders are associated with various abnormalities in platelet functions at different levels. These include: uraemia, liver disease, multiple myeloma, Waldenström's macroglobulinaemia and various myeloproliferative disorders.

COAGULATION DISORDERS

The physiology of normal coagulation is described in Chapter 17 together with relatively more common coagulation disorders of arterial and venous thrombosis and embolism. A deficiency of each of the thirteen known plasma coagulation factors has been reported, which may be inherited or acquired. In general, these coagulation disorders are uncommon as compared with other bleeding disorders. The type of bleeding in coagulation disorders is different from that seen in vascular and platelet abnormalities. Instead of spontaneous appearance of petechiae and purpuras, the plasma coagulation defects manifest more often in the form of large ecchymoses, haematomas and bleeding into muscles, joints, body cavities, GIT and urinary tract. For establishing the diagnosis, *screening tests for coagulation* (whole blood coagulation time, bleeding time,

activated partial thromboplastin time and prothrombin time) are carried out, followed by *coagulation factor assays*.

Disorders of plasma coagulation factors may have hereditary or acquired origin.

HEREDITARY COAGULATION DISORDERS. Most of the inherited plasma coagulation disorders are due to qualitative or quantitative defect in a single coagulation factor. Though defect of all the thirteen coagulation factors are reported, two of the most common inherited coagulation disorders are the sex-(X)-linked disorders—*classic haemophilia or haemophilia A* (due to inherited deficiency of factor VIII), and *Christmas disease or haemophilia B* (due to inherited deficiency of factor IX). Another common and related coagulation disorder, *von Willebrand's disease* (due to inherited defect of von Willebrand's factor), is also discussed here.

ACQUIRED COAGULATION DISORDERS. The acquired coagulation disorders, on the other hand, are usually characterised by deficiencies of multiple coagulation factors. The most common acquired clotting abnormalities are: vitamin K deficiency, coagulation disorder in liver diseases, fibrinolytic defects and disseminated intravascular coagulation (DIC).

The more common of the hereditary and acquired coagulation disorders are discussed below.

Classic Haemophilia (Haemophilia A)

Classic haemophilia or haemophilia A is the second most common hereditary coagulation disorder next to von Willebrand's disease occurring due to deficiency or reduced activity of factor VIII (anti-haemophilic factor). The disorder is inherited as a sex-(X-) linked recessive trait and, therefore, manifests clinically in males, while females are usually the carriers. However, occasional women carriers of haemophilia may produce factor VIII levels far below 50% and become symptomatic carriers, or rarely there may be true female haemophiles arising from consanguinity within the family (i.e. homozygous females). The chances of a proven carrier mother passing the abnormality onto her children is 50:50 for each son and 50:50 for each daughter. A haemophilic father will have normal sons as they inherit his Y chromosome only which does not carry the genetic abnormality.

The disease has been known since ancient times but Schönlein in 1839 gave this bleeder's disease its present name haemophilia. In 1952, it was found that haemophilia was not always due to deficiency of factor VIII as was previously thought but instead blood of some

patients was deficient in factor IX (Christmas factor or plasma thromboplastin component). Currently, *haemophilia A* (*classic haemophilia*) is the term used for the disorder due to factor VIII deficiency, and *haemophilia B* (*Christmas disease*) for the disorder when factor IX is deficient.

The frequency of haemophilia varies in different races, the highest incidence being in populations of Britain, Northern Europe and Australia. Western literature reports give an overall incidence of haemophilia in 1 in 10,000 male births. Another interesting facet of the haemophilia which has attracted investigators and researchers is the occurrence of this disorder in the royal blood of Great Britain and some European royal families.

PATHOGENESIS. Haemophilia A is caused by quantitative reduction of factor VIII in 90% of cases, while 10% cases have normal or increased levels of factor VIII with reduced activity. Factor VIII is synthesised in hepatic parenchymal cells and regulates the activation of factor X in intrinsic coagulation pathway. Factor VIII circulates in blood complexed to another larger protein, von Willebrand's factor (vWF), which comprises 99% of the factor VIII-vWF complex. The genetic coding, synthesis and functions of vWF are different from those of factor VIII and are considered separately below under von Willebrand's disease. Normal haemostasis requires 25% factor VIII activity. Though occasional patients with 25% factor VIII level may develop bleeding, most symptomatic haemophilic patients have factor VIII levels below 5%.

CLINICAL FEATURES. Patients of haemophilia suffer from bleeding for hours or days after the injury. The clinical severity of the disease correlates well with plasma level of factor VIII activity. Haemophilic bleeding can involve any organ but occurs most commonly as recurrent painful haemarthroses and muscle haematomas, and sometimes as haematuria. Spontaneous intracranial haemorrhage and oropharyngeal bleeding are rare, but when they occur they are the most feared complications.

Symptomatic patients with bleeding episodes are treated with factor VIII replacement therapy, consisting of factor VIII concentrates or plasma cryoprecipitates. With the availability of this treatment, the life expectancy of even severe haemophilic patients was approaching normal but the occurrence of AIDS in multitransfused haemophilic patients has adversely affected the life expectancy.

LABORATORY FINDINGS. The following tests are abnormal:

1. Whole blood coagulation time is prolonged in severe cases only.
2. Prothrombin time is usually normal.
3. Activated partial thromboplastin time (APTT or PTTK) is typically prolonged.
4. Specific assay for factor VIII shows lowered activity. The diagnosis of *female carriers* is made by the findings of about half the activity of factor VIII, while the *manifest disease* is associated with factor VIII activity below 25%.

Christmas Disease (Haemophilia B)

Inherited deficiency of factor IX (Christmas factor or plasma thromboplastin component) produces Christmas disease or haemophilia B. Haemophilia B is rarer than haemophilia A; its estimated incidence is 1 in 100,000 male births. The inheritance pattern and clinical features of factor IX deficiency are indistinguishable from those of classic haemophilia but accurate laboratory diagnosis is critical since haemophilia B requires treatment with different plasma fraction. The usual screening tests for coagulation are similar to those in classic haemophilia but bioassay of factor IX reveals lowered activity.

The therapy in symptomatic haemophilia B consists of infusion of either fresh frozen plasma or a plasma enriched with factor IX. Besides the expected possibilities of complications of hepatitis, chronic liver disease and AIDS, the replacement therapy in factor IX deficiency may activate the coagulation system and cause thrombosis and embolism.

von Willebrand's Disease

DEFINITION AND PATHOGENESIS. von Willebrand's disease (vWD) is the most common hereditary coagulation disorder occurring due to qualitative or quantitative defect in von Willebrand's factor (vWF). Its incidence is estimated to be 1 in 1,000 individuals of either sex. The vWF comprises the larger fraction of factor VIII-vWF complex which circulates in the blood. Though the two components of factor VIII-vWF complex circulate together as a unit and perform the important function in clotting and facilitate platelet adhesion to subendothelial collagen, vWF differs from factor VIII in the following respects:

1. The *gene* for vWF is located at chromosome 12, while that of factor VIII is in X-chromosome. Thus, vWD is inherited as an autosomal dominant trait which may

occur in either sex, while factor VIII deficiency (haemophilia A) is a sex (X)-linked recessive disorder.

2. The vWF is *synthesised* in the endothelial cells, megakaryocytes and platelets but not in the liver cells, while the principal site of synthesis of factor VIII is the liver.
3. The main *function* of vWF is to facilitate the adhesion of platelets to subendothelial collagen, while factor VIII is involved in activation of factor X in the intrinsic coagulation pathway.

CLINICAL FEATURES. Clinically, the patients of vWD are characterised by spontaneous bleeding from mucous membranes and excessive bleeding from wounds. There are 3 major types of vWD:

Type I disease is the most common and is characterised by mild to moderate decrease in plasma vWF (50% activity). The synthesis of vWF is normal but the release of its multimers is inhibited.

Type II disease is much less common and is characterised by normal or near normal levels of vWF which is functionally defective.

Type III disease is extremely rare and is the most severe form of the disease. These patients have no detectable vWF activity and may have sufficiently low factor VIII levels.

Bleeding episodes in vWD are treated with cryoprecipitates or factor VIII concentrates.

LABORATORY FINDINGS. These are:

1. Prolonged bleeding time.
2. Normal platelet count.
3. Reduced plasma vWF concentration.
4. Defective platelet aggregation with ristocetin, an antibiotic.
5. Reduced factor VIII activity.

Vitamin K Deficiency

Vitamin K is a fat-soluble vitamin which plays important role in haemostasis since it serves as a cofactor in the formation of 6 prothrombin complex proteins (*vitamin K-dependent coagulation factors*) synthesised in the liver: factor II, VII, IX, X, protein C and protein S. Vitamin K is obtained from green vegetables, absorbed in the small intestine and stored in the liver. Some quantity of vitamin K is endogenously synthesised by the bacteria in the colon.

Vitamin K deficiency may present in the newborn or in subsequent childhood or adult life:

■ **Neonatal vitamin K deficiency.** Deficiency of vitamin K in the newborn causes haemorrhagic disease of the newborn. Liver cell immaturity, lack of gut bacterial synthesis of the vitamin and low quantities in breast milk, all contribute to vitamin K deficiency in the newborn and may cause haemorrhage on 2nd to 4th day of life. Routine administration of vitamin K to all newly born infants has led to disappearance of neonatal vitamin K deficiency.

■ **Vitamin K deficiency in children and adult.** There are 3 major causes of vitamin K deficiency in childhood or adult life:

1. Inadequate dietary intake.
2. Intestinal malabsorption.
3. Loss of storage site due to hepatocellular disease.

With the onset of vitamin K deficiency, the plasma levels of all the 6 vitamin K-dependent factors (prothrombin complex proteins) fall. This, in turn, results in prolonged PT and PTTK. Parenteral administration of vitamin K rapidly restores vitamin K levels in the liver.

Coagulation Disorders in Liver Disease

Since liver is the major site for synthesis and metabolism of coagulation factors, liver disease often leads to multiple haemostatic abnormalities. The liver also produces inhibitors of coagulation such as antithrombin III and protein C and S and plays a role in the clearance of activated factors and fibrinolytic enzymes. Thus, patients with liver disease may develop hypercoagulability and are predisposed to develop DIC and systemic fibrinolysis.

The major causes of bleeding in liver diseases are as under:

I. Anatomic lesions. These include:

1. Portal hypertension e.g. varices, splenomegaly with secondary thrombocytopenia.
2. Peptic ulceration.
3. Gastritis.

II. Hepatic dysfunctions, e.g.

1. Impaired hepatic synthesis of coagulation factors.
2. Impaired hepatic synthesis of coagulation inhibitors: protein C, protein S and antithrombin III.
3. Impaired absorption and metabolism of vitamin K.
4. Failure to clear activated coagulation factors causing DIC and systemic fibrinolysis.

III. Complications of therapy. These causes are:

1. Following massive transfusion leading to dilution of platelets and coagulation factors.
2. Infusion of activated coagulation proteins.

3. Following heparin therapy.

Many a times, the haemostatic abnormality in liver disease is complex but most patients have prolonged PT and PTTK, mild thrombocytopenia, normal fibrinogen level and decreased hepatic stores of vitamin K.

Fibrinolytic Defects

Normally, fibrinolysis consisting of plasminogen-plasmin and fibrin degradation products (FDPs) is an essential protective physiologic mechanism to limit the blood coagulation in the body. However, unchecked and excessive fibrinolysis may sometimes be the cause of bleeding. The causes of *primary pathologic fibrinolysis* leading to haemorrhagic defects are:

1. Deficiency of α_2 -plasmin inhibitor following trauma or surgery.
2. Impaired clearance of tissue plasminogen activator such as in cirrhosis of liver.

At times, it may be difficult to distinguish primary pathologic fibrinolysis from secondary fibrinolysis accompanying DIC.

DISSEMINATED INTRAVASCULAR COAGULATION (DIC)

Disseminated intravascular coagulation (DIC), also termed defibrination syndrome or consumption coagulopathy, is a complex thrombo-haemorrhagic disorder (intravascular coagulation and haemorrhage) occurring as a secondary complication in some systemic diseases.

ETIOLOGY. Although there are numerous conditions associated with DIC, most frequent causes are listed below:

1. **Massive tissue injury:** in obstetrical syndromes (e.g. abruptio placentae, amniotic fluid embolism, retained dead foetus), massive trauma, metastatic malignancies, surgery.
2. **Infections:** especially endotoxaemia, gram-negative and meningococcal septicaemia, certain viral infections, malaria, aspergillosis.
3. **Widespread endothelial damage:** in aortic aneurysm, haemolytic-uraemic syndrome, severe burns, acute glomerulonephritis.
4. **Miscellaneous:** snake bite, shock, acute intravascular haemolysis, heat stroke.

PATHOGENESIS. Although in each case, a distinct triggering mechanism has been identified, the sequence of events, in general, can be summarised as under (Fig. 35.7):

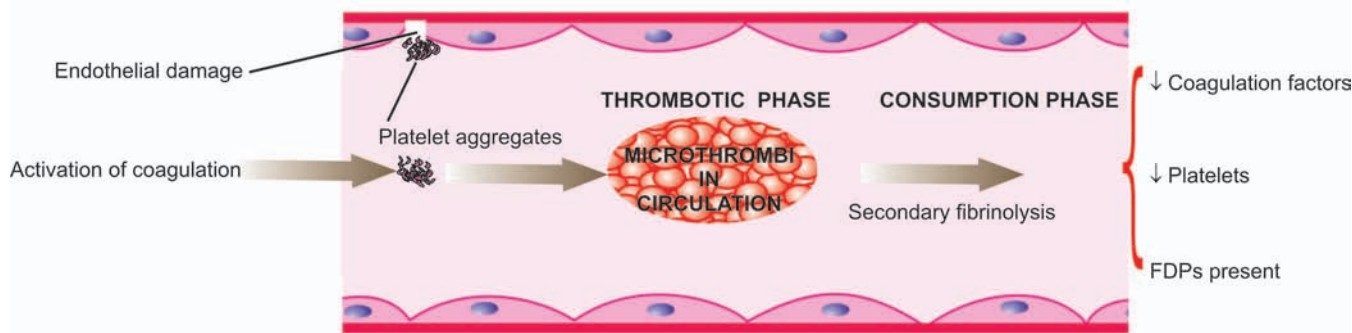


FIGURE 35.7

The pathogenesis of disseminated intravascular coagulation.

1. Activation of coagulation. The etiologic factors listed above initiate widespread activation of coagulation pathway by release of tissue factor.

2. Thrombotic phase. Endothelial damage from the various thrombogenic stimuli causes generalised platelet aggregation and adhesion with resultant deposition of

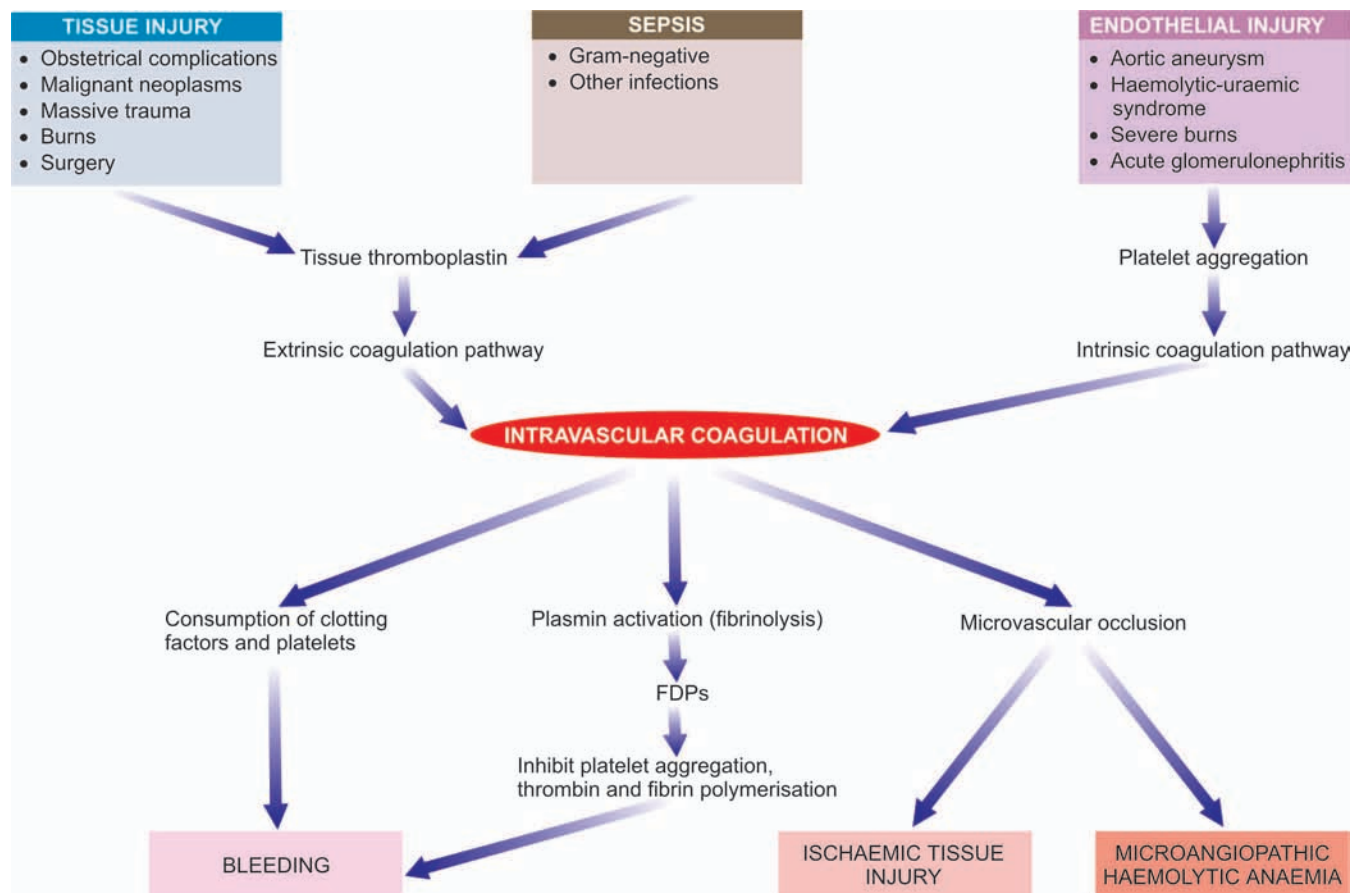


FIGURE 35.8

Pathophysiology of disseminated intravascular coagulation.

small thrombi and emboli throughout the microvasculature.

3. Consumption phase. The early thrombotic phase is followed by a phase of consumption of coagulation factors and platelets.

4. Secondary fibrinolysis. As a protective mechanism, fibrinolytic system is secondarily activated at the site of intravascular coagulation. Secondary fibrinolysis causes breakdown of fibrin resulting in formation of FDPs in the circulation.

Pathophysiology of DIC is summed up schematically in Fig. 35.8.

CLINICAL FEATURES. There are 2 main features of DIC—*bleeding* as the most common manifestation, and *organ damage* due to ischaemia caused by the effect of widespread intravascular thrombosis such as in the kidney and brain. Less common manifestations include:

microangiopathic haemolytic anaemia and thrombosis in larger arteries and veins.

LABORATORY FINDINGS. The laboratory manifestations include the following:

1. The platelet count is low.
2. Blood film shows the features of microangiopathic haemolytic anaemia. There is presence of schistocytes and fragmented red cells due to damage caused by trapping and passage through the fibrin thrombi.
3. Prothrombin time, thrombin time and activated partial thromboplastin time, are all prolonged.
4. Plasma fibrinogen levels are reduced due to consumption in microvascular coagulation.
5. Fibrin degradation products (FDPs) are raised due to secondary fibrinolysis.



Blood Groups and Blood Transfusion

BLOOD GROUP ANTIGENS AND ANTIBODIES

The term *blood group* is applied to any well-defined system of red blood cell antigens which are inherited characteristics. Over 20 blood group systems having approximately 400 blood group antigens are currently recognised. The ABO and Rhesus (Rh) blood group systems are of major clinical significance. Other minor and clinically less important blood group systems are: Lewis system, P system, I system, MNS system, Kell and Duffy system, and Luthern system.

Individuals who lack the corresponding antigen and have not been previously transfused have *naturally-occurring antibodies* in their serum. The most important are anti-A and anti-B antibodies, usually of IgM class. *Immune antibodies*, on the other hand, are acquired in response to transfusion and by transplacental passage during pregnancy. These are warm antibodies, usually of IgG class.

ABO SYSTEM. This system consists of 3 major allelic genes: A, B and O, located on the long arm of chromosome 9. These genes control the synthesis of blood group antigens A and B. The serum of an individual contains naturally-occurring antibodies to A and/or B antigen, whichever antigen is lacking in the person's red cells (Table 36.1). Two subgroups of A— A_1 and A_2 , and thus of AB also, A_1B and A_2B , are recognised but are of minor clinical significance. In routine practice, the ABO type is determined by testing the red blood cells with anti-A and anti-B and by testing the serum against A, B and O red blood cells.

Red blood cells of type O and A_2 have large amounts of another antigen called *H substance* which is genetically different from ABO but is a precursor of A and B antigens. An O group individual who inherits A or B

genes but fails to inherit H gene from either parent is called O_h *phenotype* or *Bombay blood group*. In such rare individual, despite the presence of all the three antibodies in serum (anti-A, anti-B and anti-H), the red cells are not agglutinated by the antisera.

RHESUS SYSTEM. The Rhesus (Rh) blood group system was first discovered on human red cells by the use of antisera prepared by immunising rabbits with red cells from a Rhesus monkey. The Rh allelic genes are *C* or *c*, *D* or *d* and *E* or *e*, located on chromosome 1. One set of 3 genes is inherited from each parent giving rise to various complex combinations. The corresponding antigens are similarly named *CcEe* and only *D* since no *d* antigen exists.

However, out of all these, *D* antigen is most strongly immunogenic and, therefore, clinically most important. In practice, Rh grouping is performed with anti-D antiserum. Individuals who are D-positive are referred to as *Rh-positive* and those who lack D antigen are termed *Rh-negative*.

Practically, there are no naturally-occurring Rh antibodies. All Rh antibodies in Rh-negative individuals are acquired from immunisation such as by transfusion and during pregnancy, resulting in fatal haemolytic transfusion reaction and haemolytic disease of the newborn (described later).

BLOOD TRANSFUSION

A pre-transfusion compatibility testing is essential prior to any blood transfusion. The procedure consists of the following:

1. ABO and Rh(D) grouping of the patient (*recipient*).
2. *Antibody screening* of the patient's serum to detect the presence of clinically significant antibodies.
3. Selecting the *donor* blood of the same ABO and Rh group.
4. *Cross-matching* the patient's serum against donor red cells to confirm donor-recipient compatibility.

The indications for blood transfusion are acute blood loss and various haematologic disorders considered already. In addition to the whole blood transfusion, the modern blood-banking techniques have made it possible to transfuse blood components such as packed red blood

TABLE 36.1: The ABO Blood Groups.

BLOOD GROUP	ANTIGENS ON RED CELLS	NATURALLY-OCCURRING SERUM ANTIBODIES
AB	AB	None
A	A	Anti-B
B	B	Anti-A
O	O	Anti-A, Anti-B

cells, platelets, white blood cell concentrates, plasma components and plasmapheresis in specific situations.

Complications of Blood Transfusion

A carefully prepared and supervised blood transfusion is quite safe. However, in 5-6% of transfusions, untoward complications occur, some of which are minor while others are more serious and at times fatal.

Transfusion reactions are generally classified into 2 types: *immune and non-immune*.

I. *Immunologic transfusion reactions* may be against red blood cells (haemolytic reactions), leucocytes, platelets or immunoglobulins.

II. *Non-immune transfusion reactions* include circulatory overload, massive transfusion, or transmission of an infectious agent.

These transfusion reactions are considered below.

I. IMMUNOLOGIC TRANSFUSION REACTIONS. These are as under:

1. Haemolytic transfusion reactions. Haemolytic transfusion reaction may be *immediate* or *delayed*, *intravascular* or *extravascular*.

■ The very rapid cell destruction associated with *intravascular haemolysis* is usually due to ABO incompatibility since both naturally-occurring antibodies, anti-A and anti-B, are capable of fixing complement. The symptoms include restlessness, anxiety, flushing, chest or lumbar pain, tachypnoea, tachycardia and nausea, followed by shock and renal failure.

■ *Extravascular haemolysis* is more often due to immune antibodies of the Rh system. The clinical manifestations are relatively less severe and usually consist of malaise and fever but shock and renal failure may rarely occur. Some patients develop delayed reactions in which the patient develops anaemia due to destruction of red cells in the RE system about a week after transfusion. Such delayed reactions are generally the result of previous transfusion or pregnancy (anamnestic reaction).

2. Other allergic reactions. Besides haemolytic transfusion reaction, others are:

i) *Febrile reaction* which is usually attributed to immunologic reaction against white blood cells, platelets, or IgA class immunoglobulins.

ii) Patients with antibodies against IgA molecule sometimes develop *anaphylactic shock* on transfusion of blood from other human subjects.

iii) *Allergic reactions* such as urticaria may occur.

iv) Transfusion-related *graft-versus-host disease* mediated by donor T lymphocytes may occur.

II. NONIMMUNE TRANSFUSION REACTIONS. This category includes the following adverse effects:

1. Circulatory overload. Circulatory overload resulting in pulmonary congestion and acute heart failure is the most important and most common complication that may result in death following transfusion. The risk of circulatory overload is particularly high in patients with chronic anaemia, and in infants and the elderly. The onset may be immediate, or may be delayed up to 24 hours.

2. Massive transfusion. When the volume of stored blood transfused to bleeding patients exceeds their normal blood volume, it results in dilutional thrombocytopenia and dilution of coagulation factors.

3. Transmission of infection. Many diseases can be transmitted by transfusion of an infected blood. These include: hepatitis (HBV, HCV), CMV infection, syphilis, malaria, toxoplasmosis, infectious mononucleosis, brucellosis and AIDS (HIV infection). The incidence increases in patients who receive multiple transfusions such as cases of haemophilia, thalassaemia major, acute leukaemias, acute severe haemorrhage etc. It is, therefore, mandatory that before transfusion, every unit of blood is screened for the serologic testing of HIV, HBV, HCV and syphilis and for the presence of malarial parasite.

4. Air embolism. Air embolism is unlikely to occur if the blood transfusion is carried out with plastic bags with negative pressure as is the usual practice now-a-days. A debilitated person may develop symptomatic air embolism even if a small volume (10-40 ml) makes its way into the circulation, whereas a healthy individual is at lesser risk.

5. Thrombophlebitis. The complication of thrombophlebitis is more commonly associated with venesection for blood transfusion, especially if it is done in the saphenous vein of the ankle rather than the veins of the arm. The risk of developing thrombophlebitis is further enhanced if the transfusion is continued longer than 12 hours at a single site.

6. Transfusion haemosiderosis. Post-transfusion iron overload with deposition of iron in the tissues of the body occurs after repeated transfusions in the absence of any blood loss e.g. in thalassaemia major and in severe chronic refractory anaemias. The body has no other means of getting rid of extra iron except iron excretion at the rate of 1 mg per day. A unit of whole blood

(400 ml) contains about 250 mg of iron. After approximately 100 units, the liver, myocardium and endocrine glands are all damaged.

BLOOD COMPONENTS

Blood from donors is collected as whole blood in a suitable anticoagulant. Now-a-days it is a common practice to divide whole blood into components which include: packed RBCs, platelets, fresh-frozen plasma (FFP) and cryoprecipitate.

The procedure consists of initial centrifugation at low speed to separate whole blood into two parts: *packed RBCs* and *platelet-rich plasma (PRP)*. Subsequently, PRP is centrifuged at high speed to yield two parts: *random donor platelets* and FFP. *Cryoprecipitates* are obtained by thawing of FFP followed by centrifugation. *Apheresis* is the technique of direct collection of large excess of platelets from a single donor.

The applications of these blood components in clinical use is briefly given below.

1. Packed RBCs. These are used to raise the oxygen-carrying capacity of blood and are used in normovolaemic patients of anaemia without cardiac disease. One unit of packed RBCs may raise haemoglobin by 1 g/dl.

2. Platelets. Transfusion of platelets is done in patients of thrombocytopenia who have haemorrhage. Optimally, platelet transfusions can be given to a patient with platelet count below 10,000/ μ l. Each unit of platelets can raise platelet count by 5,000 to 10,000/ μ l.

3. Fresh frozen plasma. FFP contains plasma proteins and coagulation factors that includes albumin, protein C and S and antithrombin. FFP transfusion is indicated in patients of coagulation failure and TTP. Each unit of FFP raises coagulation factors by about 2%.

4. Cryoprecipitate. Cryoprecipitate is a source of insoluble plasma proteins, fibrinogen, factor VIII and vWF. Indications for transfusion of cryoprecipitate are for patients requiring fibrinogen, factor VIII and vWF. Transfusion of single unit of cryoprecipitate yields about 80 IU of factor VIII.

HAEMOLYTIC DISEASE OF NEWBORN

Haemolytic disease of the newborn (HDN) results from the passage of IgG antibodies from the maternal circulation across the placenta into the circulation of

the foetal red cells. Besides pregnancy, sensitisation of the mother may result from previous abortions and previous blood transfusion.

HDN can occur from incompatibility of ABO or Rh blood group system. ABO incompatibility is much more common but the HDN in such cases is usually mild, while Rh-D incompatibility results in more severe form of the HDN.

PATHOGENESIS. The pathogenesis of the two main forms of HDN is different.

HDN due to Rh-D incompatibility. Rh incompatibility occurs when a Rh-negative mother is sensitised to Rh-positive blood. This results most often from a Rh-positive foetus by passage of Rh-positive red cells across the placenta into the circulation of Rh-negative mother. Normally, during pregnancy very few foetal red cells cross the placenta but haemorrhage during parturition causes significant sensitisation of the mother. Sensitisation is more likely if the mother and foetus are ABO compatible rather than ABO incompatible. Though approximately 95% cases of Rh-HDN are due to anti-D, some cases are due to combination of anti-D with other immune antibodies of the Rh system such as anti-C and anti-E, and rarely anti-c alone.

It must be emphasised here that the risk of sensitisation of a Rh-negative woman married to Rh-positive man is small in first pregnancy but increases during successive pregnancies if prophylactic anti-D immunoglobulin is not given within 72 hours after the first delivery. If both the parents are Rh-D positive (homozygous), all the newborns will be Rh-D positive, while if the father is Rh-D positive (heterozygous), there is a 50% chance of producing a Rh-D negative child.

HDN due to ABO incompatibility. About 20% pregnancies with ABO incompatibility between the mother and the foetus develop the HDN. Naturally-occurring anti-A and anti-B antibodies' which are usually of IgM class do not cross the placenta, while immune anti-A and anti-B antibodies which are usually of IgG class may cross the placenta into foetal circulation and damage the foetal red cells. ABO HDN occurs most frequently in infants born to group O mothers who possess anti-A and/or anti-B IgG antibodies. ABO-HDN differs from Rh(D)-HDN, in that it occurs in first pregnancy, Coombs' (antiglobulin) test is generally negative, and is less severe than the latter.



The lymphoid system tissues consisting of peripheral lymphoid organs (lymph nodes, spleen, mucosa-associated lymphoid tissue—MALT, pharyngeal lymphoid tissue) and thymus are closely interlinked with the function of the bone marrow. B and T lymphocytes formed after differentiation from lymphopoietic precursor cells in the bone marrow undergo further maturation in peripheral lymphoid organs and thymus respectively. The discussion below pertains to diseases pertaining to lymph nodes and spleen.

LYMPH NODES

NORMAL STRUCTURE

The lymph nodes are bean-shaped or oval structures varying in length from 1 to 2 cm and form the part of lymphatic network distributed throughout the body. Each lymph node is covered by a connective tissue *capsule*. At the convex surface of the capsule several *afferent lymphatics* enter which drain into the peripheral *subcapsular sinus*, branch into the lymph node and terminate at the concavity (hilum) as a single *efferent lymphatic* vessel. These lymphatic vessels are lined by mononuclear phagocytic cells.

The inner structure of the lymph node is divided into a peripheral cortex and central medulla. The *cortex* consists of several rounded aggregates of lymphocytes called *lymphoid follicles*. The follicle has a pale-staining germinal centre surrounded by small dark-staining lymphocytes called the mantle zone. The deeper region of the cortex or *paracortex* is the zone between the peripheral cortex and the inner medulla. The *medulla* is predominantly composed of cords of plasma cells and some lymphocytes. The capsule and the structure within the lymph node are connected by supportive delicate reticulin framework (Fig. 37.1,A).

Functionally, the lymph node is divided into T and B lymphocyte zones:

- *B-cell zone* lies in the follicles in the cortex, the mantle zone and the interfollicular space, while *plasma cells* are also present in the interfollicular zone.
- *T-cell zone* is predominantly present in the medulla.

There are two main functions of the lymph node—to mount immune response in the body, and to perform the function of active phagocytosis for particulate material. Besides T and B-cells, the follicular centre has *dendritic histiocytes* and antigen presenting *Langerhans'*

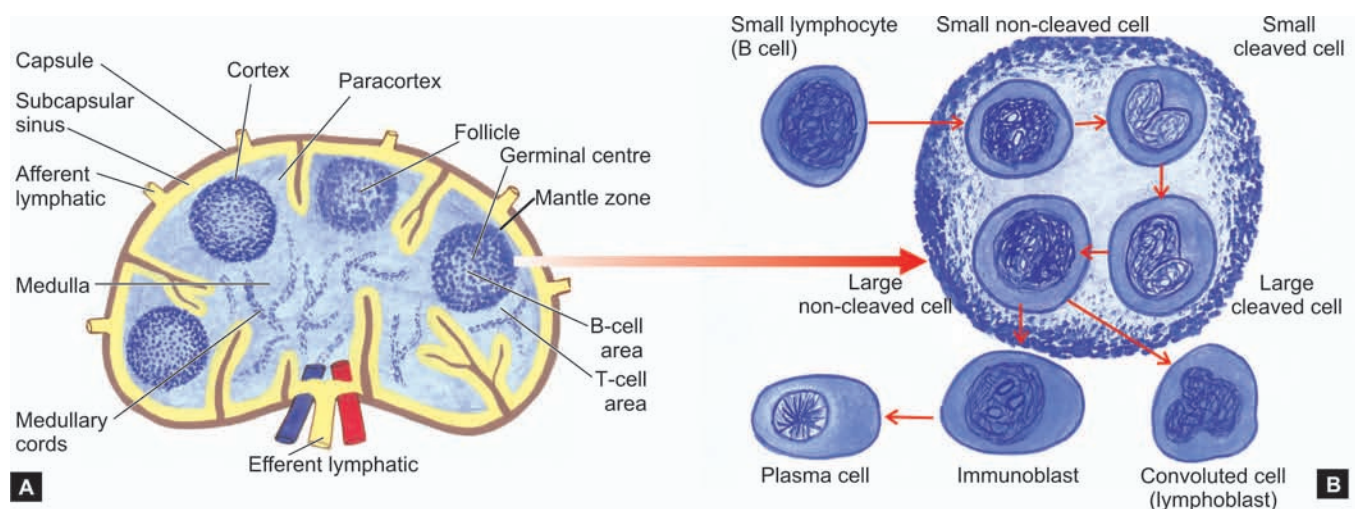


FIGURE 37.1

A, The anatomic structure and functional zones of a lymph node. B, Maturation of lymphoid cells in the follicle.

histiocytes (formerly together called tingible body macrophages due to engulfment of particulate material) and *endothelial cells*. The follicular centre is a very active zone where lymphocytes from peripheral blood continuously enter and leave, interact with macrophage-histiocytes and endothelial cells and undergo maturation and transformation. Lymphocytes and endothelial cells have surface molecules which interact and serve as 'addresses' so that endothelial cells can direct the lymphocytes; these molecules are appropriately termed as *addressins* or *homing receptors*. Peripheral blood B and T lymphocytes on entering the lymph node are stimulated immunologically which transforms them to undergo cytoplasmic and nuclear maturation which may be in the follicular centre or paracortex as per following sequence and schematically depicted in Fig. 37.1.B:

- i) Follicular centre, small non-cleaved cells or centroblasts
- ii) Follicular centre, small cleaved cells or centrocytes
- iii) Follicular centre, large cleaved cells
- iv) Follicular centre, large non-cleaved cells
- v) Immunoblasts (in paracortex)
- vi) Convoluted cells or lymphoblasts (in paracortex)
- vii) Plasma cells

Lymph nodes are secondarily involved in a variety of systemic diseases, local injuries and infections, and are also the site for some important primary neoplasms. Many of these diseases such as tuberculosis, sarcoidosis, histoplasmosis, typhoid fever, viral infections etc have been considered elsewhere in the textbook alongwith description of these primary diseases. In the section of diseases of the lymph node that follows, the following topics are discussed:

1. Reactive lymphadenitis.
2. Malignant lymphomas.
3. Lymph node metastatic tumours.
4. Plasma cell disorders.
5. Langerhans' cell histiocytosis.

REACTIVE LYMPHADENITIS

Lymph nodes undergo reactive changes in response to a wide variety of stimuli which include microbial infections, drugs, environmental pollutants, tissue injury, immune-complexes and neoplasia. However, the most common causes of lymph node enlargement are inflammatory and immune reactions, aside from primary malignant neoplasms and metastatic tumour deposits. Those due to primary inflammatory reaction are termed *reactive lymphadenitis*, and those due to primary immune reactions are referred to as *lymphadenopathy*.

Reactive lymphadenitis is a nonspecific response and is categorised into acute and chronic types. In addition, a few variant lymphadenitis are also described below.

Acute Nonspecific Lymphadenitis

All kinds of acute inflammations may cause acute nonspecific lymphadenitis in the nodes draining the area of inflamed tissue. Most common causes are microbiologic infections or their breakdown products, and foreign bodies in the wound or into the circulation etc. Most frequently involved lymph nodes are: *cervical* (due to infections in the oral cavity), *axillary* (due to infection in the arm), *inguinal* (due to infection in the lower extremities), and *mesenteric* (due to acute appendicitis, acute enteritis etc).

Acute lymphadenitis is usually mild and transient but occasionally it may be more severe. Acutely inflamed nodes are enlarged, tender, and if extensively involved, may be fluctuant. The overlying skin is red and hot. After control of infection, majority of cases heal completely without leaving any scar. If the inflammation does not subside, acute lymphadenitis changes into chronic lymphadenitis.

PATHOLOGIC CHANGES. *Grossly*, the affected lymph nodes are enlarged 2-3 times their normal size and may show abscess formation if the involvement is extensive.

Microscopically, the sinusoids are congested, widely dilated and oedematous and contain numerous neutrophils. The lymphoid follicles are prominent with presence of many mitoses and phagocytosis. In more severe cases, necrosis may occur and neutrophil abscesses may form.

Chronic Nonspecific Lymphadenitis

Chronic nonspecific lymphadenitis, commonly called *reactive lymphoid hyperplasia*, is a common form of inflammatory reaction of draining lymph nodes as a response to antigenic stimuli such as repeated attacks of acute lymphadenitis and lymph from malignant tumours.

Depending upon the pattern in chronic nonspecific lymphadenitis, three types are distinguished, each having its own set of causes. These are: *follicular hyperplasia*, *paracortical hyperplasia* and *sinus histiocytosis*. However, mixed patterns may also be seen in which case one of the patterns predominates over the others.

PATHOLOGIC CHANGES. *Grossly*, the affected lymph nodes are usually enlarged, firm and non-tender.

Microscopically, the features of 3 patterns of reactive lymphoid hyperplasia are as under:

1. **Follicular hyperplasia** is the most frequent pattern, particularly encountered in children. Besides nonspecific stimulation, a few specific causes are:

rheumatoid arthritis, toxoplasmosis, syphilis and AIDS. The microscopic features are as follows:

- i) There is marked enlargement and prominence of the germinal centres of lymphoid follicles (proliferation of B-cell areas) due to the presence of numerous mitotically active lymphocytes and proliferation of phagocytic cells containing phagocytosed material.
- ii) Parafollicular and medullary regions are more cellular and contain plasma cells, histiocytes, and some neutrophils and eosinophils.
- iii) There is hyperplasia of mononuclear phagocytic cells lining the lymphatic sinuses in the lymph node.

■ *Angiofollicular lymphoid hyperplasia* or *Castleman's disease* is a clinicopathologic variant of follicular hyperplasia. The condition may occur at any age and possibly has an association with Epstein-Barr virus infection. Two histologic forms are distinguished:

- i) *Hyaline-vascular type* is more common (90% cases) and is characterised by the presence of hyalinised arterioles in small lymphoid follicles and proliferation of vessels in the interfollicular area.
- ii) *Plasma cell form* is less common and is characterised by plasma cell hyperplasia and vascular proliferation in the interfollicular region.

2. Paracortical lymphoid hyperplasia is due to hyperplasia of T-cell-dependent area of the lymph node. Amongst the important causes are immunologic reactions caused by drugs (e.g. dilantin), vaccination, viruses (e.g. infectious mononucleosis) and autoimmune disorders. Its histologic features are:

- i) Expansion of the paracortex (T-cell area) with increased number of T-cell transformed immunoblasts.
- ii) Encroachment by the enlarged paracortex on the lymphoid follicles, sometimes resulting in their effacement.
- iii) Hyperplasia of the mononuclear phagocytic cells in the lymphatic sinuses.

Variants of paracortical lymphoid hyperplasia are angio-immunoblastic lymphadenopathy, dermatopathic lymphadenopathy, dilantin lymphadenopathy and post-vaccinal lymphadenopathy.

■ *Angioimmunoblastic lymphadenopathy* is characterised by diffuse hyperplasia of immunoblasts rather than paracortical hyperplasia only, and there is proliferation of blood vessels. The condition occurs in elderly patients with generalised lymph node enlargement and hypergammaglobulinaemia.

■ *Dermatopathic lymphadenopathy* occurs in lymph node draining an area of skin lesion. Besides the

hyperplastic paracortex, there is presence of dark melanin pigment within the macrophages in the lymph node.

3. Sinus histiocytosis or sinus hyperplasia is a very common type found in regional lymph nodes draining inflammatory lesions, or as an immune reaction of the host to a draining malignant tumour or its products. The hallmark of histologic diagnosis is the expansion of the sinuses by proliferating large histiocytes containing phagocytosed material. The presence of sinus histiocytosis in the draining lymph nodes of carcinoma such as in breast carcinoma has been considered by some workers to confer better prognosis in such patients due to good host immune response.

■ *Sinus histiocytosis with massive lymphadenopathy* is characterised by marked enlargement of lymph nodes, especially of the neck, in young adolescents. It is associated with characteristic clinical features of painless but massive lymphadenopathy with fever and leucocytosis and usually runs a benign and self-limiting course.

HIV-related Lymphadenopathy

HIV infection and AIDS have already been discussed in Chapter 13; here one of the frequent finding in early cases of AIDS, **persistent generalised lymphadenopathy (PGL)**, is described. The presence of enlarged lymph nodes of more than 1 cm diameter at two or more extra-inguinal sites for more than 3 months without any other obvious cause is frequently the earliest symptom of primary HIV infection.

Microscopically, the findings at biopsy of involved lymph node vary depending upon the stage of HIV infection:

1. *In the early stage* marked follicular hyperplasia is the dominant finding and reflects the polyclonal B-cell proliferation.
2. *In the intermediate stage*, there is a combination of follicular hyperplasia and follicular involution. However, adenopathic form of Kaposi's sarcoma too may develop at this stage.
3. *In the last stage*, there is decrease in the lymph node size indicative of prognostic marker of disease progression. Microscopic findings of node at this stage reveal follicular involution and lymphocyte depletion. At this stage, other stigmata of AIDS in the lymph node may also appear e.g. lymphoma, mycobacterial infection, toxoplasmosis, systemic fungal infections etc.

MALIGNANT LYMPHOMAS

Lymphomas are malignant tumours of lymphoreticular origin i.e. from lymphocytes and histiocytes and their precursor cells. Clinically and pathologically, lymphomas are quite heterogeneous. However, two distinct clinicopathologic groups are routinely distinguished:

I. *Hodgkin's lymphoma* or *Hodgkin's disease (HD)* characterised by pathognomonic presence of Reed-Sternberg cells. This group comprises about 25% of all cases of malignant lymphomas.

II. *Non-Hodgkin's lymphomas (NHL)* are more common and comprise the rest of cases.

Both these groups have further histopathologic subtypes and are considered separately below.

HODGKIN'S DISEASE

Hodgkin's disease (HD) primarily arises within the lymph nodes and involves the extranodal sites secondarily. The incidence of the disease has bimodal peaks—one in young adults between the age of 15 and 35 years and the other peak after 5th decade of life. The HD is more prevalent in young adult males than females. The classical diagnostic feature is the presence of *Reed-Sternberg (RS) cell* (or *Dorothy-Reed-Sternberg cell*) (described later).

Etiopathogenesis

The nature and origin of RS cells, which are the real neoplastic cells in HD, have been a matter of considerable debate. One main reason for this difficulty in their characterisation is that in HD, unlike most other malignancies, the number of neoplastic cells (i.e. RS cells) is very small (less than 5%) which are interspersed in the predominant reactive cells. However, it is still not clear what stimulates these lymphoid cells to undergo transformation. The following possible hypotheses are suggested:

1. **EBV.** For years, Epstein-Barr virus (EBV) has been implicated in the etiology of HD on the basis of epidemiologic and serologic studies. Recently, on the basis of molecular studies, genome of EBV has been identified in majority of cases of mixed cellularity type and some cases of nodular sclerosis HD.
2. **Genetic etiology.** It is suggested on the basis of observation of occurrence of HD in families and with certain HLA type. HD is 99 times more common in identical twin of an affected case compared with general population, implicating genetic origin strongly.
3. **Cytokines.** Presence of reactive inflammatory cells in the HD is due to secretion of cytokines from the RS cells e.g. IL-5 (growth factor for eosinophils), IL-13 (for

autocrine stimulation of RS cells) and transforming growth factor- β (for fibrogenesis).

Classification

The diagnosis of HD requires accurate microscopic diagnosis by biopsy, usually from lymph node, and occasionally from other tissues. Unlike NHL, there is only one universally accepted classification of HD i.e. *Rye classification* adopted since 1966. Rye classification divides HD into the following 4 subtypes:

1. Lymphocyte-predominance type.
2. Nodular-sclerosis type.
3. Mixed-cellularity type.
4. Lymphocyte-depletion type.

More recently, however, according to the new WHO classification of all malignancies of lymphoid tissues (Harris et al, 1999) which takes into account clinical, morphologic, immunologic and genetic information, HD has been divided into 2 main groups:

- I. Nodular lymphocyte-predominant HD (a new type).
- II. Classic HD (includes all the 4 above subtypes in the Rye classification).

Central to the diagnosis of HD is the essential identification of *Reed-Sternberg cell* though this is not the sole criteria (see below).

The salient features of the 4 histologic subtypes of HD are summarised in Table 37.1.

Reed-Sternberg Cell

The diagnosis of Hodgkin's disease rests on identification of RS cells, though uncommonly similar cells can occur in infectious mononucleosis and other forms of lymphomas. Therefore, additional cellular and architectural features of the biopsy must be given due consideration for making the histologic diagnosis.

There are several morphologic variants of RS cells which characterise different histologic subtypes of HD (Fig. 37.2):

1. **Classic RS cell** is a large cell which has characteristically a bilobed nucleus appearing as mirror image of each other but occasionally the nucleus may be multi-lobed. Each lobe of the nucleus contains a prominent, eosinophilic, inclusion-like nucleolus with a clear halo around it, giving an owl-eye appearance. The cytoplasm of cell is abundant and amphophilic.
2. **Lacunar type RS cell** is smaller and in addition to above features has a pericellular space or lacuna in which it lies, which is due to artefactual shrinkage of the cell cytoplasm. It is characteristically found in nodular sclerosis variety of HD.

TABLE 37.1: Modified Rye Classification of Hodgkin's Disease.

HISTOLOGIC SUBTYPE	INCIDENCE	MAIN PATHOLOGY	RS CELLS	PROGNOSIS
I. CLASSIC HD				
<i>Lymphocyte-predominance</i>	5%	Proliferating lymphocytes, a few histiocytes	Few, classic and polyploid type, CD15-, CD30-, CD20+	Excellent
<i>Nodular sclerosis</i>	70%	Lymphoid nodules, collagen bands	Frequent, lacunar type, CD15+, CD30+	Very good
<i>Mixed cellularity</i>	22%	Mixed infiltrate	Numerous, classic type, CD15+, CD30+	Good
<i>Lymphocyte-depletion (Diffuse fibrotic and reticular variants)</i>	1%	Scanty lymphocytes, atypical histiocytes, fibrosis	Numerous, pleomorphic type, CD15+, CD30+	Poor
II. NODULAR LYMPHOCYTE-PREDOMINANT HD				
	2%	Proliferation of small lymphocytes, nodular pattern of growth	Sparse number of RS cells, CD45+, EMA+, CD15-, CD30-	Chronic relapsing, may transform into large B cell NHL

3. Polyploid type (or popcorn or lymphocytic-histiocytic i.e. L and H) RS cells are seen in lymphocyte predominance type of HD. This type of RS cell is larger with lobulated nucleus in the shape of popcorn.

4. Pleomorphic RS cells are a feature of lymphocyte depletion type. These cells have pleomorphic and atypical nuclei.

It may be mentioned here that in general the number of RS cells is inversely proportional to the number of lymphocytes in a particular histologic subtype of HD.

Immunophenotyping of RS cells reveals monoclonal lymphoid cell origin of RS cell from B-cells in most subtypes of Hodgkin's disease. RS cells in all types of Hodgkin's diseases, except in lymphocyte predominance type, express immunoreactivity for CD15 and CD30. RS cells in lymphocyte predominance type, however, are negative for both CD15 and CD30, but positive for CD20.

RS cells are invariably accompanied by variable number of *atypical Hodgkin cells* which are believed to be precursor RS cells but are not considered diagnostic of HD. Hodgkin cells are large mononuclear cells (rather than mirror image nuclei) having nuclear and cytoplasmic similarity to that of RS cell.

PATHOLOGIC CHANGES. *Grossly*, the affected lymph nodes initially remain discrete but later are matted together. The sectioned surface is homogeneous and fishflesh-like in *lymphocyte predominance type*, nodular due to scarring in *nodular sclerosis type*, and abundance of necrosis in *mixed cellularity* and *lymphocyte depletion types*. Hodgkin's disease of the

liver, spleen and other organs forms spherical masses similar to metastatic carcinoma.

Microscopically, the criteria for diagnosis of histologic subtypes are as under:

I. CLASSIC HD

As per WHO classification, classic group of HD includes 4 types of HD of older Rye classification:

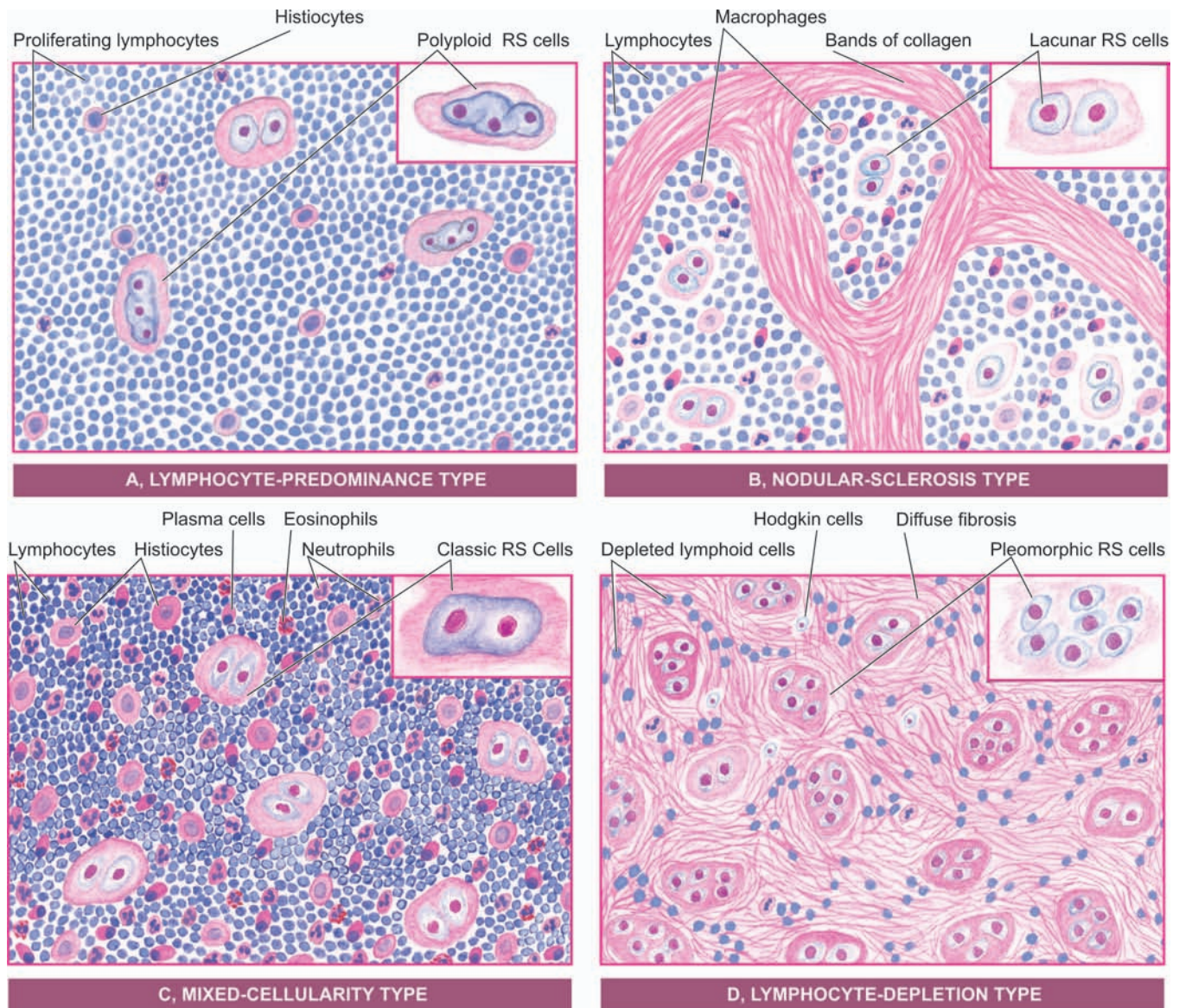
1. Lymphocyte-predominance type (Fig. 37.2,A). The lymphocyte-predominance type of HD is characterised by proliferation of small lymphocytes admixed with a varying number of histiocytes forming nodular or diffuse pattern.

i) *Nodular form* is characterised by replacement of nodal architecture by numerous large neoplastic nodules.

ii) *Diffuse form* does not have discernible nodules but instead there is diffuse proliferation of cells.

However, currently nodular form of lymphocyte predominant HD has been categorised separately due to its distinct immunophenotyping features and prognosis (discussed below).

For making the diagnosis, definite demonstration of RS cells is essential which are few in number, requiring a thorough search. In addition to typical RS cells, *polyploid variant* having polyploid, and twisted nucleus (popcorn-like) may be found in some cases. This type of HD usually does not show other cells like plasma cells, eosinophils and neutrophils, nor are necrosis or fibrosis seen.

**FIGURE 37.2**

Microscopic features of 4 forms of Hodgkin's disease of lymph node. The inset on right side of each type shows the morphologic variant of RS cell seen more often in particular histologic type.

2. Nodular-sclerosis type (Fig. 37.2,B). Nodular sclerosis is the most frequent type of HD, seen more commonly in women than in men. It is characterised by two essential features:

- i) *Bands of collagen*: Variable amount of fibrous tissue is characteristically present in the involved lymph nodes. Occasionally, the entire lymph node may be replaced by dense hyalinised collagen.
- ii) *Lacunar type RS cells*: Characteristic lacunar type of RS cells with distinctive pericellular halo are

present. These cells appear lacunar due to the shrinkage of cytoplasm in formalin-fixed tissue. The pericellular halo is not seen if the tissue is fixed in Zenker's fluid.

In addition to these 2 characteristics, the nodules between the fibrous septa consist predominantly of lymphocytes and macrophages, sometimes with foci of necrosis.

3. Mixed-cellularity type (Fig. 37.2,C). This form of HD generally replaces the entire affected lymph

nodes by heterogeneous mixture of various types of apparently normal cells. These include proliferating lymphocytes, histiocytes, eosinophils, neutrophils and plasma cells. Some amount of fibrosis and focal areas of necrosis are generally present. Typical RS cells are frequent (COLOUR PLATE XI: CL 44).

4. Lymphocyte-depletion type (Fig. 37.2,D). In this type of HD, the lymph node is depleted of lymphocytes. There are two variants of lymphocyte-depletion HD:

- i) *Diffuse fibrotic variant* is hypocellular and the entire lymph node is replaced by diffuse fibrosis, appearing as homogeneous, fibrillar hyaline material. The area of hyalinosis contains some lymphocytes, atypical histiocytes (Hodgkin cells), and numerous typical and atypical (pleomorphic) RS cells.
- ii) *Reticular variant* is much more cellular and consists of large number of atypical pleomorphic histiocytes, scanty lymphocytes and a few typical RS cells.

II. NODULAR LYMPHOCYTE-PREDOMINANT HD

This is a newly described entity which is distinct from the classic HD described above. This type was previously included in lymphocyte predominant type of HD. Its peculiarities are as under:

- i) These cases of HD have a nodular growth pattern (similar to nodular sclerosis type).
- ii) Like lymphocyte-predominant pattern of classic type, there is predominance of small lymphocyte with sparse number of RS cells.
- iii) These cases of HD have distinctive immunophenotyping: CD45 positive, epithelial membrane (EMA) positive but negative for the usual markers for RS cells (CD15 and CD30 negative).
- iv) Though generally it has a chronic relapsing course, but some cases of this type of HD may transform in to large B-cell NHL.

Clinical Features

Hodgkin's disease is particularly frequent among young and middle-aged adults. All histologic subtypes of HD, except the nodular sclerosis variety, are more common in males. The disease usually begins with superficial lymph node enlargement and subsequently spreads to other lymphoid and non-lymphoid structures.

1. Most commonly, patients present with painless, movable and firm *lymphadenopathy*. The cervical and mediastinal lymph nodes are involved most frequently. Other lymph node groups like axillary, inguinal and abdominal are involved sometimes.

2. Approximately half the patients develop *splenomegaly* during the course of the disease. *Liver enlargement* too may occur.

3. *Constitutional symptoms* (type B symptoms) are present in 25-40% of patients. The most common is low-grade fever with night sweats and weight loss. Other symptoms include fatigue, malaise, weakness and pruritus.

Other Laboratory Findings

Besides clinical and pathologic findings, there are some haematologic and immunologic abnormalities in HD.

Haematologic abnormalities:

1. A moderate, normocytic and normochromic *anaemia* is often present.
2. *Serum iron and TIBC* are low but marrow iron stores are normal or increased.
3. *Marrow infiltration* by the disease may produce marrow failure with leucoerythroblastic reaction.
4. *Routine blood counts* reveal moderate leukaemoid reaction. Cases with pruritus frequently show peripheral eosinophilia. Advanced disease is associated with absolute lymphopenia.
5. *Platelet count* is normal or increased.
6. *ESR* is invariably elevated.

Immunologic abnormalities:

1. There is progressive fall in immunocompetent T-cells with *defective cellular immunity*. There is reversal of CD4: CD8 ratio and anergy to routine skin tests.
2. *Humoral antibody production* is normal in untreated patients until late in the disease.

Staging

Following biopsy and histopathologic classification of HD, the extent of involvement of the disease (i.e. staging) is studied so as to select the proper treatment and assess the prognosis. *The Ann Arbor staging classification* takes into account both clinical and pathologic stage of the disease.

The suffix *A* or *B* are added to the above stages depending upon whether the three constitutional symptoms (fever, night sweats and unexplained weight loss exceeding 10% of normal) are absent (*A*) or present (*B*). The suffix *E* or *S* are used for extranodal involvement and splenomegaly respectively (Table 37.2).

For complete staging, a number of other *essential diagnostic studies* are recommended. These are as under:

TABLE 37.2: Ann Arbor Staging Classification of Hodgkin's Disease.

Stage I	I	Involvement of a single lymph node region.
(A or B)	I_E	Involvement of a single extra-lymphatic organ or site.
Stage II	II	Involvement of two or more lymph node regions on the same side of the diaphragm.
(A or B)	II_E	(or) with localised contiguous involvement of an extranodal organ or site.
Stage III	III	Involvement of lymph node regions on both sides of the diaphragm.
(A or B)	III_E	(or) with localised contiguous involvement of an extranodal organ or site.
	III_S	(or) with involvement of spleen.
	III_{ES}	(or) both features of III _E and III _S .
Stage IV	IV	Multiple or disseminated involvement of one or more extra-lymphatic organs or tissues with or without lymphatic involvement.
(A or B)		

A = asymptomatic; B = presence of constitutional symptoms;
E = extranodal involvement; S = splenomegaly

1. Detailed physical examination including sites of nodal involvement and splenomegaly.
2. Chest radiograph to exclude mediastinal, pleural and lung parenchymal involvement.
3. CT scan of abdomen and pelvis.
4. Documentation of constitutional symptoms (B symptoms).
5. Laboratory evaluation of complete blood counts, liver and kidney function tests.
6. Bilateral bone marrow biopsy.
7. Finally, histopathologic documentation of the type of Hodgkin's disease.

More invasive investigations include *lymphangiography of lower extremities* and *staging laparotomy*. Staging laparotomy includes biopsy of selected lymph nodes in the retroperitoneum, splenectomy and wedge biopsy of the liver.

Prognosis

With use of aggressive radiotherapy and chemotherapy, the outlook for Hodgkin's disease has improved significantly. Although several factors affect the prognosis, two important considerations in evaluating its outcome are the *extent of involvement by the disease (i.e. staging)* and the *histologic subtype*.

■ With appropriate treatment, the overall 5 years survival rate for *stage I and II A* is as high as about 100%, while the advanced stage of the disease may have upto 50% 5-year survival rate.

■ Patients with *lymphocyte-predominance type* of HD tend to have localised form of the disease and have excellent prognosis.

■ The *nodular sclerosis* variety too has very good prognosis but those patients with larger mediastinal mass respond poorly to both chemotherapy and radiotherapy.

■ The *mixed cellularity type* occupies intermediate clinical position between the lymphocyte predominance and the lymphocyte-depletion type, but patients with disseminated disease and systemic manifestations do poorly.

■ The *lymphocyte-depletion type* is usually disseminated at the time of diagnosis and is associated with constitutional symptoms. These patients usually have the most aggressive form of the disease.

NON-HODGKIN'S LYMPHOMAS

Non-Hodgkin's lymphomas (NHL) are the malignant neoplasms of the immune system of the body and are more common than Hodgkin's lymphoma. The biologic and clinical behaviour of NHL are quite distinct from HD and thus the two are quite different diseases. NHL is most frequent in young adults (20-40 years). Its incidence is showing an upward trend due to increasing incidence of AIDS.

Majority of NHL arise in lymph nodes (65%) while the remaining 35% take origin in extranodal lymphoid tissues. However, all forms of NHL have potential to spread to other lymph nodes, liver, spleen and bone marrow. The involvement of bone marrow may be followed by spill over of NHL into the peripheral blood, and vice versa when lymphoid leukaemia originating in the bone marrow may spread to lymph nodes, creating an overlapping picture of lymphoid leukaemia and NHL. In such situations, it may become difficult to determine which appeared first in the affected patient.

Etiopathogenesis

Malignant lymphomas are considered as the clonal proliferation of immune cells. About 65% of NHL are of B-lymphocyte origin, 35% of T-lymphocytes, and less than 2% are histiocytic derivatives. The agents involved in inducing neoplastic transformation of these cells are poorly understood, but the following etiologic factors have been shown to have strong association with the development of NHL.

1. Infections. There is evidence to suggest that certain infections are involved in development of lymphomas:

i) *Viral infections:* Epstein-Barr virus (EBV) in endemic variety of Burkitt's lymphoma and post-transplant

lymphoma, human-T-cell leukaemia virus type I (HTLV-I) in adult T-cell lymphoma-leukaemia, HIV in diffuse large B-cell lymphoma and Burkitt's lymphoma, hepatitis C virus in lymphoplasmacytic lymphoma, and human herpes virus 8 (HHV-8) in primary effusion lymphoma.

ii) *Bacteria: Helicobacter pylori* in MALT lymphoma of the stomach.

2. Immunodeficiency diseases. Various inherited and acquired immunodeficiency diseases including AIDS and iatrogenic immunosuppression induced by chemotherapy or radiation, are associated with subsequent development of lymphomatous transformation.

3. Autoimmune disease association. A few autoimmune diseases such as Sjögren's syndrome, non-tropical sprue, rheumatoid arthritis and SLE are associated with higher incidence of NHL.

4. Chemical and drug exposure. Long-term exposure to drugs such as phenytoin, radiation, prior chemotherapy and agriculture chemicals have all been associated with development of NHL. Patients treated for HD can develop NHL.

A number of cytogenetic abnormalities have been detected in cases of NHL, most important of which are chromosomal translocations involving antigen receptor genes, immunoglobulin genes, or overexpression of *BCL-2* protein.

Classification

Unlike Hodgkin's disease in which Rye classification is the only universally accepted classification since 1966 with some modifications, the pathologic classification of NHL has been difficult for both pathologists and clinicians. Several classifications have been described at different times adding further confusion. The widely used classification systems of NHL (in chronologic order) are: *Rappaport* (1966), *Lukes-Collins* (1974), *Working Formulations for clinical usage* (1982), *REAL* (1994), and *WHO classification* (1999). These classifications are given in Table 37.3 and briefly discussed below.

RAPPAPORT CLASSIFICATION. Rappaport in 1966 proposed a clinically relevant pathologic classification based on the following two main features:

1. *Low-power microscopy* of the overall pattern of the lymph node architecture.
2. *High-power microscopy* revealing the cytology of the neoplastic cells.

Based on these two features, Rappaport divided NHL into two major subtypes:

1. *Nodular or follicular lymphomas* which retain some of the features of normal lymph node in that the neoplastic cells form lymphoid 'nodules' rather than lymphoid follicles with germinal centres.

2. *Diffuse lymphomas*, on the other hand, are characterised by effacement of the normal lymph node architecture and there may be infiltration of neoplastic cells outside the capsule of the involved lymph node.

NHL was further classified according to the degree of differentiation of neoplastic cells into: *well-differentiated*, *poorly-differentiated*, and *histiocytic (large cells) types* of both nodular and diffuse lymphomas.

However, with the advent of modern immunologic methods, the following objections were raised regarding the applicability of Rappaport classification:

1. The term histiocytic lymphoma was found incorrect since almost all lymphomas were of lymphoid origin.
2. With the identification of T and B-cells and their subpopulations made possible, the Rappaport classification was found to be incomplete as regards the cell of origin of different subtypes of NHL.

LUKES-COLLINS CLASSIFICATION. Lukes and Collins in 1974 proposed a classification correlating the type of NHL with the immune system. Yet another modification of Lukes-Collins called *Kiel classification* appeared in 1981.

Employing immunologic markers for tumour cells, Lukes-Collins divided all malignant lymphomas into either B-cell or T-cell origin and rarely macrophages. The B and T-cell tumours are further subdivided on the basis of their light microscopic characteristics. The majority of NHL are B lymphocyte derivatives (about 65%) and arise from follicular centre cells (FCC). The FCC in the germinal centre undergo transformation to become large immunoblasts and pass through the four stages—*small cleaved cells* and *large cleaved cells*, *small non-cleaved cells* and *large non-cleaved cells*.

Though Lukes-Collins classification is immunologically correct, the classification is unclear about varying prognosis of different clinical types of NHL of either B-cell or T-cell origin.

WORKING FORMULATIONS FOR CLINICAL USAGE. This classification proposed in 1982 by a panel of experts from National Cancer Institute of the US incorporates the best features of all previous classification systems, and as the name implies, has strong clinical relevance. Based on the natural history of disease and long-term survival studies, Working Formulations divides all NHLs into 3 prognostic groups:

- *Low-grade NHL*: 5-year survival 50-70%;

- *Intermediate-grade NHL*: 5-year survival 35-45%; and
- *High-grade NHL*: 5-year survival 25-35%.

In this classification, no attempt is made to determine whether the tumour cells are B-cells, T-cells or macrophages. Each prognostic group includes a few morphologic subtypes, and lastly, a miscellaneous group is also described.

REAL CLASSIFICATION. International Lymphoma Study Group in 1994 has proposed another classification called *revised European-American classification of lymphoid neoplasms* abbreviated as REAL classification. This classification is based on the hypothesis that all forms of lymphoid malignancies (NHLs as well as lymphoblastic leukaemias) represent malignant counterparts of normal population of immune cells (B-cells, T-cells and histiocytes) present in the lymph node and bone marrow. It is believed that lymphoid malignancies arise due to arrest at the various differentiation stages of B and T-cells since tumours of histiocytic origin are quite uncommon. Accordingly, it is considered essential to understand and correlate the differentiation stages of B and T-cells with various lymphoid malignancies (Fig. 37.3). REAL classification divides all lymphoid malignancies into two broad groups, each having further subtypes (Table 37.3):

- *Leukaemias and lymphomas of B-cell origin*: B-cell derivation comprises 80% cases of lymphoid leukaemias and 90% cases of NHLs. Based upon these phenotypic and genotypic features, B-cell neoplasms are of pre-B and mature B-cell origin. Based on their biologic behaviour, B-cell malignancies are further subclassified into indolent and aggressive. All these tumours express Pan-B (CD19) antigen besides other markers.

- *Leukaemias and lymphomas of T-cell origin*: T-cell malignancies comprise the remainder 20% cases of lymphoid leukaemia and 10% cases of NHLs. T-cell malignancies reflect the stages of T-cell ontogeny. Like B-cell malignancies, T-cell derivatives too are further categorised into indolent and aggressive T-cell malignancies. The most widely expressed T-cell antigens are CD2 and CD7.

WHO CLASSIFICATION. In 1999, the same group of workers (Harris et al) who described REAL classification revised their classification under the aegis of WHO. Although this classification has many similarities with REAL classification as regards identification of B and T cell types (Fig. 37.3), WHO classification has more classes. Besides, it takes into consideration cytogenetic and molecular profile of all lymphoid malignancies and has a better clinical and therapeutic relevance.

PATHOLOGIC CHANGES

The diagnosis of NHL can only be reliably made on examination of lymph node biopsy.

Grossly, the affected group of lymph nodes are enlarged in majority of cases of NHL. Any lymph node group may be involved but most commonly affected are the cervical, supraclavicular and axillary groups. Less commonly at the time of diagnosis, there may be enlargement of lymphoid tissue elsewhere such as in the tonsils, spleen, stomach, bone etc. Initially, the lymph nodes are discrete and separate from one another but later the lymph nodes form a large matted mass due to infiltration into the surrounding connective tissue. Extranodal involvements produce either a discrete tumour or diffuse enlargement of the affected organ. The sectioned surface of the involved lymph nodes or extranodal organ involved appears grey-white and fishflesh-like. Thus, the gross appearance of Hodgkin's and non-Hodgkin's lymphoma is much the same.

Microscopically, it is beyond the scope of the present text to describe the morphological details of all the subtypes of NHL listed in Table 37.3 and Fig. 37.3 for which the reference works may be consulted. However, salient features of common clinicopathologic subtypes as per recent WHO classification are briefly outlined below. The synonyms in brackets in each subgroup of NHL described below (wherever counterpart subtypes applicable) correspond to the Rappaport, Lukes-Collins, and Working Formulation for Clinical Usage classification respectively.

I. B-CELL NEOPLASMS

PRECURSOR B-CELL MALIGNANCIES

Precursor B-cell lymphocytic leukaemia-lymphoma (*Diffuse lymphoblastic; Convolutated T-cell lymphoma; Lymphoblastic lymphoma*). This is the most common form of ALL in children; rarely presentation may be in the form of lymphoma in children or adults. The diagnosis is made by bone marrow biopsy which shows malignant cells of precursor B cell origin as demonstrated by immunophenotyping (CD10, 19, 20, 22, HLA-DR and TdT positive) and characteristic cytogenetic abnormalities, most often t(9;22) i.e. Philadelphia positive-ALL.

The clinical features of profound bone marrow involvement such as pallor, fatigue, bleeding and infections due to cytopenia are present. Peripheral blood generally shows anaemia and thrombocytopenia, and

	NORMAL B-CELL DIFFERENTIATION	MARKERS	B-CELL MALIGNANCIES	NORMAL T-CELL DIFFERENTIATION	MARKERS	T-CELL MALIGNANCIES	
BONE MARROW		CD10, 19, 20, 22 HLA-DR+, TdT			CD2, 7, 38, 71, TdT		
	Pre-B cell		Pre-B ALL	Stage I prothymocyte		T-ALL (majority)	
LYMPHOID FOLLICLE		CD19, 20, 22, 21, 5 HLA-DR+			CD1, 2, 4, 7, 8, 38		THYMUS
	Mantle zone B-cell		Mantle cell NHL, B-CLL/ SLL	Stage II thymocyte		T-ALL (majority) T-NHL (some)	
		CD19, 20, 21, 22 HLA-DR+			CD2, 3, 4, 5, 6, 7		
FCC		CD19, 20, 21, 22 HLA-DR+			CD2, 3, 4, 5, 6, 7		LN AND PERIPHERAL BLOOD
	Mature B-cell		Follicular NHL, Diffuse NHL	Mature T helper cell		T-cell (majority) T-NHL (Sezary LL, cutaneous NHL)	
		CD38, 19, 20, PCA-1+			CD2, 3, 4, 5, 6, 7 TCR		
	Secretory B-cell (Plasma cell)		Myeloma, Waldenström's	Mature T suppressor cell		T-cell LL (some) T-cell NHL (some)	

(PCA- Plasma cell antigen; TCR = T-cell receptor; FCC = Follicular centre B- cells, LN = Lymph node, LL = Lymphoid leukaemia)

FIGURE 37.3

Schematic representation of WHO-REAL classification. Various immunophenotypes of B and T-cell malignancies are correlated with normal immunophenotypic differentiation/maturation stages of B and T-cells in the bone marrow, lymphoid tissue, peripheral blood and thymus.

sometimes leucopenia. In cases having leukaemic presentation, extranodal site involvement is early such as lymphadenopathy, hepatomegaly, splenomegaly, CNS infiltration, testicular enlargement, and at times cutaneous infiltration.

MATURE (PERIPHERAL) B-CELL MALIGNANCIES

1. B-cell CLL/SLL (Chronic lymphocytic leukaemia—small lymphocytic lymphoma). (*Diffuse, well-*

differentiated lymphocytic; B-small lymphocytic and plasmacytoid lymphoma; Small lymphocytic lymphoma). As the name implies, this subtype may present as leukaemia or lymphoma. As lymphoid leukaemia, this is the most common form, while as NHL it constitutes about 4% of all NHLs. The diagnosis of B-cell CLL is made by circulating lymphocytosis in excess of 4,000/ μ l with typical smudge or basket cells

TABLE 37.3: Classification of NHL.

RAPPAPORT CLASSIFICATION (1966)**A) Nodular NHL**

1. Lymphocytic, well-differentiated
2. Lymphocytic, poorly-differentiated
3. Lymphocytic and histiocytic, mixed

B) Diffuse NHL

1. Lymphocytic, well-differentiated
2. Lymphocytic, poorly-differentiated
3. Lymphocytic and histiocytic, mixed
4. Histiocytic
5. Lymphoblastic
6. Diffuse undifferentiated, Burkitt's and non-Burkitt's

LUKES-COLLINS CLASSIFICATION (1974)**A) B-cell NHL**

1. Small lymphocytic
2. Plasmacytoid lymphocytic
3. Follicular centre cell (small cleaved and large cleaved, small non-cleaved and large non-cleaved)
4. Immunoblastic

B) T-cell NHL

1. Small lymphocytic
2. Convuluted lymphocytic
3. Cerebriform lymphocytic
4. Immunoblastic

C) Histiocytic NHL**D) Undefined (U) NHL****WORKING FORMULATIONS FOR CLINICAL USAGE (1982)****I. Low-grade**

- A) Small lymphocytic
- B) Follicular, predominantly small cleaved cell
- C) Follicular, mixed small and large cleaved cell

II. Intermediate-grade

- D) Follicular, predominantly large cell
- E) Diffuse, small cleaved cell
- F) Diffuse, mixed small and large cell
- G) Diffuse, large, cell

III. High-grade

- H) Large cell, immunoblastic
- I) Lymphoblastic
- J) Small non-cleaved cell (Burkitt's)

IV. Miscellaneous

1. Adult T-cell leukaemia/lymphoma
2. Cutaneous T-cell lymphoma
3. Histiocytic (Histiocytic medullary reticulosis)

REAL CLASSIFICATION (1994)**I. Leukaemias and lymphomas of B-cell origin****(Pan-B CD19,20 positive)****A) Indolent B-cell malignancies**

- i) Chronic lymphocytic leukaemia/small lymphocytic lymphoma
- ii) Hairy cell leukaemia
- iii) Follicular lymphomas (grade I small cleaved, grade II mixed small and large)
- iv) Lymphoplasmacytoid lymphoma/Waldenström's macroglobulinaemia
- v) Marginal zone lymphoma (lymphoma of mucosa-associated lymphoid tissue (MALT), splenic lymphoma)

B) Aggressive B-cell malignancies

- i) Diffuse large cell lymphoma
- ii) Follicular large cell lymphoma (grade III)
- iii) Mantle cell lymphoma
- iv) Burkitt's lymphoma
- v) Plasmacytoma/myeloma

II. Leukaemias and lymphomas of T-cell origin (CD2,7 positive)**a) Indolent T-cell malignancies**

- i) T-CLL, T-prolymphocytic leukaemia
- ii) Cutaneous T-cell lymphoma (Sézary's syndrome, mycosis fungoides)

b) Aggressive T-cell malignancies

- i) Peripheral T-cell NHL
- ii) Angioimmunoblastic T-cell lymphoma
- iii) Intestinal T-cell lymphoma
- iv) Adult T-ALL

WHO CLASSIFICATION (1999)**I. B-cell neoplasms****Precursor B-cell malignancies:**

Precursor B lymphoblastic leukaemia/lymphoma (precursor B-cell ALL)

Mature (Peripheral) B-cell malignancies:

1. B-cell CLL/SLL (chronic lymphocytic leukaemia/small lymphocytic lymphoma)
2. Plasma cell myeloma/plasmacytoma (page 534)
3. Extranodal marginal zone B-cell lymphoma, MALT type
4. Mantle cell lymphoma
5. Follicular lymphoma
6. Diffuse large B-cell lymphoma
7. Burkitt's lymphoma/Burkitt cell leukaemia
8. Other B-cell malignancies:
 - i) B-cell prolymphocytic leukaemia
 - ii) Hairy cell leukaemia
 - iii) Splenic marginal zone B-cell lymphoma
 - iv) Lymphoplasmacytic lymphoma
 - v) Nodal marginal zone lymphoma (Monocytoid B-cell lymphoma)

II. T-cell neoplasms**Precursor T-cell malignancies:**

Precursor T lymphoblastic lymphoma/leukaemia (Precursor T-cell ALL)

Mature (Peripheral) T-cell malignancies:

1. Mycosis fungoides/Sézary syndrome
2. Adult T-cell lymphoma/leukaemia (HTLV-I +)
3. Anaplastic large T/null cell lymphoma, primary systemic type
4. Peripheral T-cell lymphoma, not otherwise specified
 - i) Angioimmunoblastic T-cell lymphoma
 - ii) Extranodal NK/T-cell lymphoma, nasal type
 - iii) Enteropathy-type T-cell lymphoma
 - iv) Hepatosplenic T-cell lymphoma
5. Other T-cell malignancies:
 - i) T-cell prolymphocytic leukaemia
 - ii) T-cell granular lymphocytic leukaemia
 - iii) Aggressive NK cell leukaemia

in the peripheral blood due to damaged nuclei of malignant lymphocytes. These cells are of monoclonal B-cell origin having immunologic features of mantle zone B-cells (typically positive for CD5; other markers are CD19, 20, 22 and for HLA-DR) and cytogenetic abnormalities (most commonly trisomy 12). Cases with lymphadenopathy at presentation show replacement of the lymph node by diffuse proliferation of well-differentiated, mature, small and uniform lymphocytes without any cytologic atypia or significant mitoses (Fig. 37.4,B) and having features of immunophenotype as for B-cell CLL.

B-cell CLL/SLL occurs more commonly in middle and older age groups. The condition may remain asymptomatic, or may present with nonspecific clinical features such as easy fatiguability, weight loss and anorexia. Patients frequently have hepatosplenomegaly, generalised lymphadenopathy, susceptibility to infections, autoimmune haemolytic anaemia and autoimmune thrombocytopenia. Generally, the course is indolent. However, some cases of SLL may transform into more aggressive diffuse large B-cell lymphoma, or may be associated with occurrence of an IgM monoclonal gammopathy called Waldenström's macroglobulinaemia.

2. Plasma cell myeloma/plasmacytoma. This entity is discussed separately later in this chapter.

3. Extranodal marginal zone B-cell lymphoma of MALT type. This type comprises about 8% of all NHLs. In the previous classification, it was included under SLL, but currently it is categorised separately for 2 reasons: etiologic association with *H. pylori* infection and occurrence at extranodal sites. Most frequent is gastric lymphoma of MALT type with its characteristic etiologic association with *H. pylori*; other extranodal sites for this subtype of NHL are intestine, orbit, lung, thyroid, salivary glands and CNS. Morphologically, it is characterised by diffuse infiltration by monoclonal small B lymphocytes which are negative for CD5.

Median age for this form of NHL is 60 years and often remains localised to the organ of origin but may infiltrate the regional lymph nodes. Rarely, it may be more aggressive and may metastasise, or transform into diffuse large B-cell lymphoma.

4. Mantle cell lymphoma. This subtype of NHL comprises about 6% of all NHLs. It was earlier included in SLL but has been identified as a separate subtype recently due to characteristic chromosomal translocation, t(11;14) and overexpression of *BCL-1*

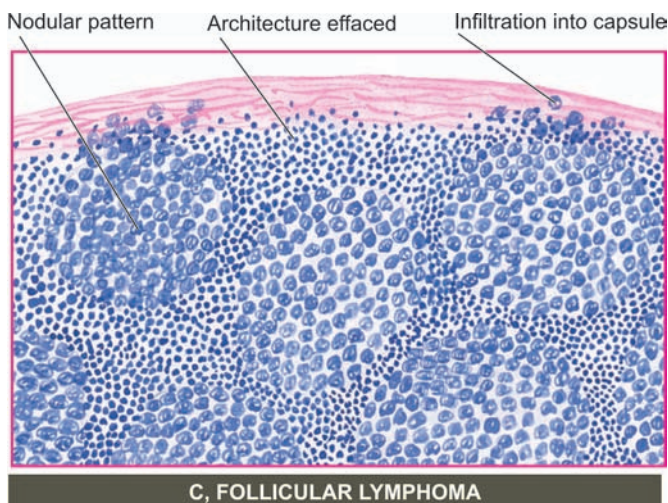
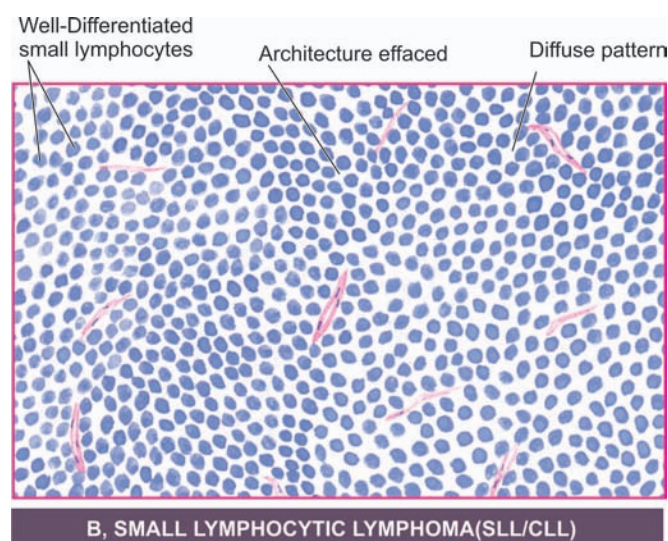
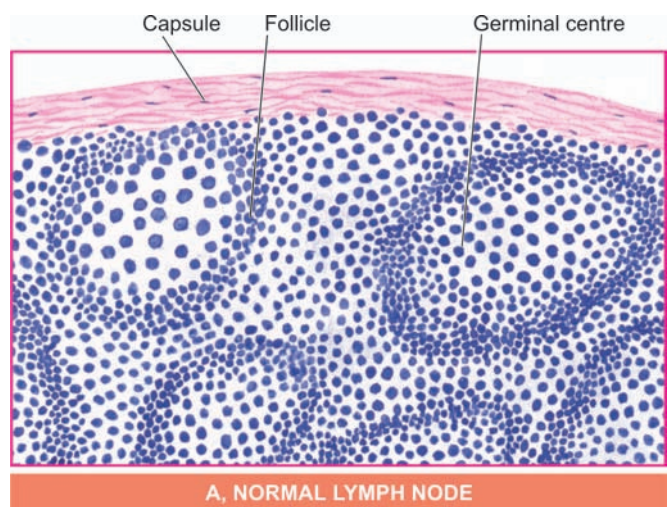


FIGURE 37.4

Prototypes of non-Hodgkin's lymphoma.

and surface immunoglobulins IgM and IgD protein. However, both SLL and mantle cell NHL are positive for CD5 antigen. Mantle cell lymphoma arises from B-cells of mantle zone of normal lymphoid follicle. The tumour cells show diffuse or nodular pattern of involvement in the lymph node and have somewhat indented nuclei.

Patients of mantle cell lymphoma are generally older males. The disease involves bone marrow, spleen, liver and bowel. It is more aggressive than other types of SLLs.

5. Follicular lymphoma (*Nodular, poorly-differentiated lymphocytic; Follicular centre cell, small/large cleaved lymphoma; Follicular, predominantly small/large cleaved cell lymphoma*). Follicular lymphomas comprise approximately 22% of all NHLs. Morphologically, as the name suggests, this form of follicular lymphoma is characterised by follicular or nodular pattern of growth. The nuclei of tumour cells may vary from predominantly small cleaved (or indented) to predominantly large cleaved variety (Fig. 37.4, C). The former is more common, has infrequent mitoses and the rate of growth slow (low grade), while the patients with large cell lymphoma have high proliferation and progress rapidly (high grade). In all follicular lymphomas, the tumour cells are positive for pan-B markers such as CD19 and CD20 along with expression of *BCL-2* protein (for distinction from normal germinal centre which is *BCL-2* negative). Cytogenetic studies show characteristic translocation t(14;18) in tumour cells.

The follicular lymphomas occur in older individuals, most frequently presenting with painless peripheral lymphadenopathy which is usually waxing and waning type. Bone marrow infiltration is typically paratrabeular. Peripheral blood involvement as occurs in SLL is uncommon in this variety. In contrast to diffuse lymphomas, extranodal involvement is also infrequent. About half the cases of low grade follicular lymphomas, during their indolent biologic course, may evolve into diffuse large B-cell lymphoma. Median survival for patients with low grade follicular lymphoma is 7-9 years.

6. Diffuse large B-cell lymphoma (*Diffuse poorly-differentiated lymphocytic/Diffuse histiocytic; Follicular centre cell, large, cleaved/non-cleaved diffuse lymphoma*). Diffuse large B-cell lymphoma is the most common comprising about 35% of all NHLs. This variety is the diffuse counterpart of follicular large cleaved cell

lymphoma i.e. it is composed of large cleaved cells spread in a diffuse pattern. The cytoplasm in these tumour cells is pale and abundant while the nuclei have prominent 1-2 nucleoli. Immunophenotypic markers for pan-B cells (CD19, CD20) are positive, besides overexpression of surface immunoglobulins (IgM, IgG and light chains) and of *BCL-2* protein.

Diffuse large B-cell lymphoma occurs in older patients with mean age of 60 years. It may present primarily as a lymph node disease or at extranodal sites. About half the cases have extranodal involvement at the time of presentation, particularly in the bone marrow and the alimentary tract. Primary diffuse large B-cell lymphoma of CNS may also occur.

A few subtypes of diffuse large B-cell lymphoma are described with distinct clinicopathologic settings:

- *Epstein-Barr virus (EBV)* infection has been etiologically implicated in diffuse large B-cell lymphoma in *immunosuppressed patients* of AIDS and organ transplant cases.

- *Human herpes virus type 8 (HHV-8)* infection along with presence of immunosuppression is associated with a subtype of diffuse large B-cell lymphoma presenting with effusion, termed *primary effusion lymphoma*.

- *Mediastinal large B-cell lymphoma* is diagnosed in patients with prominent involvement of mediastinum, occurs in young females and frequently spreads to CNS and abdominal viscera.

In general, diffuse large B-cell lymphomas are aggressive tumours and disseminate widely.

7. Burkitt's lymphoma/leukaemia (*Diffuse undifferentiated Burkitt's and Non-Burkitt's; Follicular centre cell, small non-cleaved cell lymphoma; Small non-cleaved cell lymphoma*). Burkitt's lymphoma/leukaemia is an uncommon tumour in adults but comprises about 30% of childhood NHLs. Burkitt's leukaemia corresponds to L3 ALL of FAB grouping and is uncommon. Three subgroups of Burkitt's lymphoma are recognised: *African endemic, sporadic and immunodeficiency-associated*, but histologically all three are similar. The tumour cells are intermediate in size, non-cleaved, and homogeneous in size and shape. The nuclei are round to oval and contain 2-5 nucleoli. The cytoplasm is basophilic and contains lipid vacuolation. The tumour cells have a very high mitotic rate, and therefore high cell death. This feature accounts for presence of numerous macrophages in the background of this tumour containing phagocytosed tumour debris giving it a 'starry sky' appearance (Fig. 37.5).

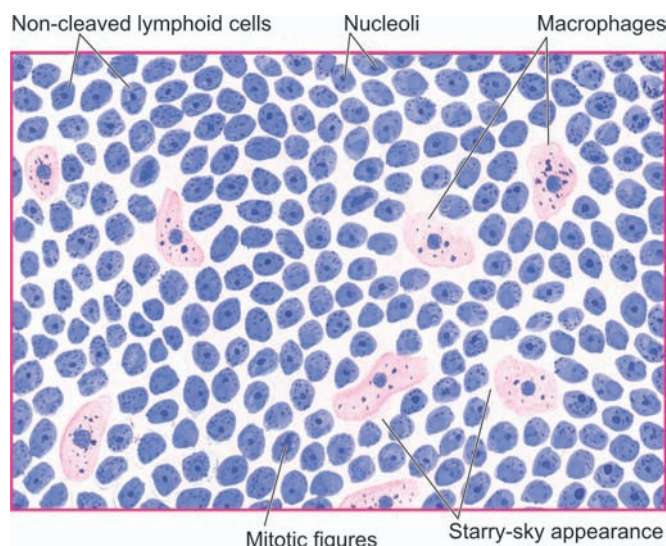


FIGURE 37.5

Burkitt's lymphoma. The tumour shows uniform cells having high mitotic rate. Scattered among the tumour cells are benign macrophages surrounded by a clear space giving 'starry sky' appearance.

■ *African endemic Burkitt's lymphoma* was first described in African children, predominantly presenting as jaw tumour that spreads to extranodal sites such as the bone marrow and meninges. The relationship of this tumour with oncogenic virus, Epstein-Barr virus (EBV), has been discussed on page 266.

■ *Sporadic Burkitt's lymphoma* is a related tumour in which the tumour cells are similar to those of Burkitt's lymphoma but are more pleomorphic and may sometimes be multinucleated. Sporadic variety has a propensity to infiltrate the CNS and is more aggressive than true Burkitt's lymphoma.

■ *Immunodeficiency-associated Burkitt's lymphoma* includes cases seen in association with HIV infection.

Immunophenotypically, the tumour cells are positive for CD19 and CD10 and surface immunoglobulin IgM. Typical cytogenetic abnormalities in the tumour cells are t(8;22) involving *MYC* gene on chromosome 8, with overexpression of *MYC* protein having transforming activity. Burkitt's lymphoma is a high grade tumour and is the most rapidly progressive human tumour.

8. Other B-cell malignancies. Brief mention is made below on the other types of B-cell malignant tumours given in the WHO classification in Table 37.3:

i) *B-cell prolymphocytic leukaemia* is involvement of blood and bone marrow by large B lymphocytes having prominent nucleoli. These patients have leucocytosis with splenomegaly and lymphadenopathy.

ii) *Hairy cell leukaemia* is a rare B-cell malignancy characterised by presence of hairy cells in the blood and bone marrow and splenomegaly. The condition has already been discussed on page 497.

iii) *Splenic marginal zone lymphoma* is another uncommon B-cell neoplasm in which the splenic white pulp is infiltrated by small monoclonal B lymphocytes. The condition has a slow and indolent behaviour.

iv) *Lymphoplasmacytic lymphoma* is tissue manifestation of Waldenström's macroglobulinaemia, discussed later in this chapter. There is infiltration by IgM-secreting monoclonal lymphoplasmacytic cells into lymph nodes, spleen, bone marrow, and sometimes in the peripheral blood. Etiologic association of this form of lymphoma with hepatitis C virus infection has also been proposed.

v) *Nodal marginal zone lymphoma (monocytoid B-cell lymphoma)* is another uncommon subtype of aggressive NHL. At presentation, the patients often have disseminated disease, involving bone marrow and leukaemic picture.

II. T-CELL NEOPLASMS PRECURSOR T-CELL MALIGNANCIES

Precursor T-cell lymphoblastic leukaemia/lymphoma. As the name implies, these cases may present as ALL or as lymphoma. Like precursor B-cell NHL, this subtype is also more common in children under 4 years of age. Since the precursor T-cells differentiate in the thymus, this tumour often presents as mediastinal mass and pleural effusion and progresses rapidly to develop leukaemia in the blood and bone marrow. Morphologically, precursor B and T-cell ALL/lymphoma are indistinguishable. However, immunophenotype of T-cell subtype is positive for CD2, CD7 and TdT.

Clinically, features of bone marrow failure are present which include anaemia, neutropenia and thrombocytopenia. Lymphadenopathy, hepatosplenomegaly and CNS involvement are frequent. Precursor T-cell lymphoma-leukaemia is, however, more aggressive than its B-cell counterpart.

MATURE (PERIPHERAL) T-CELL MALIGNANCIES

1. Mycosis fungoides/Sézary syndrome. Mycosis fungoides is a slowly evolving cutaneous T-cell lymphoma occurring in middle aged adult males. The condition is often preceded by eczema or

dermatitis for several years (*premycotic stage*). This is followed by infiltration by CD4+T-cells in the epidermis and dermis as a plaque (*plaque stage*) and eventually as *tumour stage*. The disease may spread to viscera and to peripheral blood as a leukaemia characterised by Sézary cells having cerebriform nuclei termed as Sézary syndrome.

Mycosis fungoides/Sézary syndrome is an indolent NHL and has a median survival of 8 to 9 years.

2. Adult T-cell lymphoma/leukaemia (ATLL). This is an uncommon T-cell malignancy but has gained much prominence due to association with retrovirus, human T-cell lymphotropic virus-I (HTLV-I) (page 268). The infection is acquired by blood transfusion, breast milk, sexual route or transplacentally. ATLL is observed in Japan, the Caribbean and parts of the US but is rare in rest of the world. The involved lymph nodes have proliferation of CD4 positive large atypical T-cells with indented nuclei, called 'flower cells', most prominent in the paracortical zone. The blood also shows large pleomorphic T-cell leukaemia.

The patients have usually widespread lymphadenopathy with leukaemia, hepatosplenomegaly and involvement of skin and leptomeninges. This disease has a fulminant course.

3. Anaplastic large T/Null cell lymphoma. This relatively newer entity is the T-cell counterpart of diffuse large B-cell lymphoma and was previously included under malignant histiocytosis or diagnosed as anaplastic carcinoma. There is diffuse infiltration of lymph nodes by anaplastic T-cells/null cells positive for CD30 (Ki 1). Cytogenetic abnormality consists of t(2;5). Involvement of skin occurs frequently and produces an indolent cutaneous large T/null cell lymphoma.

4. Peripheral T-cell lymphomas. This group includes a variety of aggressive T-cell lymphomas which are morphologically heterogeneous but have common immunotypic features of mature T-cells (CD4+, CD8+, or both). These are more common in young adults and often have bone marrow involvement at presentation. The subtypes of peripheral T-cell lymphomas include the following syndromes:

i) *Angioimmunoblastic T-cell lymphoma* is relatively more common subtype, comprising about 20% of all T-cell NHLs. The patients have profound constitutional symptoms (fever, weight loss, skin rash), generalised lymphadenopathy and polyclonal hypergammaglobulinaemia.

ii) *Extranodal T/NK cell lymphoma of nasal type* is involvement of upper airways by the monoclonal T-cells. The condition is quite aggressive and was earlier called as lethal midline granuloma or angiocentric lymphoma. The patients have haemophagocytic syndrome. During the course of the disease, blood and bone marrow may be involved producing leukaemic picture.

iii) *Enteropathy type T-cell lymphoma* is a rare aggressive lymphoma seen in association with untreated cases of gluten-sensitive enteropathy.

iv) *Hepatosplenic T-cell lymphoma*, unlike other lymphomas which occur as tumour masses, is characterised by sinusoidal infiltration of the liver, spleen and bone marrow by monoclonal T-cells. Systemic features are often present.

Clinical Features

Features pertaining to specific types of NHL have been given along with their morphologic description above. Some general clinical features common to most cases of NHL are as under:

1. Superficial lymphadenopathy. At presentation, there is painless, asymmetric enlargement of one or more groups of peripheral lymph nodes.

2. Constitutional symptoms. Fever, night sweats and more than 10% weight loss comprise the constitutional symptoms (type B symptoms) similar to those found in Hodgkin's disease.

3. Oropharyngeal involvement. Involvement of Waldeyer's ring is present in 5-10% of patients.

4. Abdominal disease. Enlargement of liver, spleen and mesenteric and retroperitoneal lymph nodes are found in some cases. Lymphomatous involvement of the GIT is the most common extranodal involvement after the bone marrow.

5. Other organs. Involvement of the skin or brain occurs in certain subtypes of NHL.

Other Laboratory Findings

In addition to clinical and histopathological findings, certain abnormal haematologic and immunologic findings are found in NHL.

Haematologic abnormalities:

1. Anaemia of normocytic normochromic type.
2. Advanced disease with marrow infiltration may show neutropenia, thrombocytopenia and leucoerythroblastic reaction.

3. Lymphosarcoma cell leukaemia (leukaemic conversion of NHL) occurs in some patients.
4. Marrow involvement by NHL is seen in 20% of cases.
5. Hyperuricaemia and hypercalcaemia occur late in the disease.

Immunologic abnormalities:

Immunologic studies reveal that majority of NHLs are of B lymphocyte origin. There may be associated monoclonal excess of immunoglobulins, usually IgG or IgM. Plasmacytoid lymphoma may produce excess of a heavy chain or a light chain.

Staging

The Ann Arbor staging system developed for Hodgkin's disease (page 524) is used for staging NHL too. This staging system depends upon the number and location of nodal and extranodal sites involved, and presence or absence of constitutional (B) symptoms. But the concept of staging is much less helpful in NHL than in Hodgkin's disease because the prognosis is not correlated with the anatomic site of involvement of the disease.

Prognosis

Low-grade lymphomas usually progress slowly and local radiotherapy brings about remission lasting for upto 10 years in 75% cases. More extensive disease is treated with combination chemotherapy and local radiotherapy. Children with lymphoblastic lymphoma and Burkitt's lymphoma respond extremely well to chemotherapy. Meningeal involvement is treated with irradiation of the brain. Patients with histiocytic lymphoma and T-cell leukaemia/lymphoma have an extremely poor prognosis.

The salient features to distinguish Hodgkin's disease and non-Hodgkin's lymphoma are summarised in Table 37.4.

LYMPH NODE METASTATIC TUMOURS

The regional lymph nodes draining the site of a primary malignant tumour are commonly enlarged. This enlargement may be due to benign *reactive hyperplasia* or *metastatic tumour deposits*.

1. Benign reactive hyperplasia, as already discussed (page 518), is due to immunologic reaction by the lymph node in response to tumour-associated antigens. It may be expressed as sinus histiocytosis, follicular hyperplasia, plasmacytosis and occasionally may show non-caseating granulomas.

TABLE 37.4: Contrasting Features of Hodgkin's Disease and Non-Hodgkin's Lymphoma.

FEATURE	HODGKIN'S	NON-HODGKIN'S
1. <i>Cell derivation</i>	B-cell mostly	90% B 10% T
2. <i>Nodal involvement</i>	Localised, may spread to contiguous nodes	Disseminated nodal spread
3. <i>Extranodal spread</i>	Uncommon	Common
4. <i>Bone marrow involvement</i>	Uncommon	Common
5. <i>Constitutional symptoms</i>	Common	Uncommon
6. <i>Chromosomal defects</i>	Aneuploidy	Translocations, deletions
7. <i>Spill-over</i>	Never	May spread to blood
8. <i>Prognosis</i>	Better (75-85% cure)	Bad (30-40% cure)

2. Metastatic deposits in regional lymph nodes occurs most commonly from carcinomas and malignant melanoma. Sarcomas generally disseminate by the haematogenous route but uncommonly may metastasise to the regional lymph nodes. Metastatic tumour cells from the primary malignant tumour are drained via lymphatics into the subcapsular sinuses initially but subsequently the lymph node stroma is also invaded. The pushing margins of advancing metastatic tumour in stroma of lymph node is characteristically well demarcated. Areas of necrosis are frequent in metastatic carcinomas (**COLOUR PLATE VIII: CL 31**).

The morphologic features of primary malignant tumour are recapitulated in metastatic tumour in lymph nodes.

PLASMA CELL DISORDERS

The plasma cell disorders are characterised by abnormal proliferation of immunoglobulin-producing cells and result in accumulation of monoclonal immunoglobulin in serum and urine. The group as a whole is known by various synonyms such as *plasma cell dyscrasias*, *paraproteinaemias*, *dysproteinaemias* and *monoclonal gammopathies*. The group comprises the following four disease entities:

1. Multiple myeloma
2. Waldenström's macroglobulinaemia
3. Heavy chain disease
4. Primary amyloidosis (Chapter 7).

The feature common to all plasma cell disorders is the neoplastic proliferation of cells derived from B-lymphocyte lineage. Normally B lymphocytes have surface immunoglobulin molecules of both M and G heavy chains. Under normal circumstances, the B-cells are stimulated by exposure to surface immunoglobulin-specific antigen and mature to form IgG-producing plasma cells. However, in the plasma cell disorders, the control over this process is lost and results in abnormal production of immunoglobulin that appears in the blood and urine. These disorders differ from B-cell lymphomas in having monoclonal synthesis of immunoglobulins and lack of prominent lymphadenopathy. In addition to the rise in complete immunoglobulins, some plasma cell disorders synthesise excess of light chains (kappa or lambda), or heavy chains of a single class (alpha, gamma, or mu). Bence Jones proteins are free light chains present in blood and excreted in urine of some cases with plasma cell disorders.

After these brief general comments, we can now turn to the discussion of the specific plasma cell disorders.

MULTIPLE MYELOMA

Multiple myeloma is a multifocal malignant proliferation of plasma cells derived from a single clone of cells (i.e. monoclonal). The terms multiple myeloma and myeloma are used interchangeably. The tumour, its products (M component), and the host response result in the most important and most common syndrome in the group of plasma cell disorders that produces osseous as well as extraosseous manifestations. Multiple myeloma primarily affects the elderly (peak incidence in 5th-6th decades) and increases in incidence with age. It is rare under the age of 40 and is slightly more common in males.

Etiopathogenesis

Myeloma is a monoclonal proliferation of B-cells. The etiology of myeloma remains unknown. However, following factors have been implicated:

1. Radiation exposure.
2. Those exposed to petroleum products.
3. Farmers, wood workers, leather workers are more prone.

As regards its pathogenesis, following concepts have been proposed:

1. **Genetic abnormalities.** A variety of chromosomal alterations have been observed in cases of myeloma, out of which common ones are as under:

- i) Translocation t(11;14)(q13;q32) is the most common.
- ii) Overexpression of *MYC* or *RAS* genes in some cases.
- iii) Mutation in p53 (TP53) and *RB* gene in some cases.

2. Role of growth factor. Interleukin (IL)-6 has been found to play role in B-cell proliferation in myeloma. Infection of the marrow macrophages with human herpes virus 8 with resultant release of viral IL-6 may be responsible for stimulation of neoplastic process in myeloma.

Pathologic Changes

Myeloma affects principally the bone marrow though in the course of the disease other organs are also involved. Therefore, the pathologic findings are described below under two headings—*osseous (bone marrow) lesions* and *extraosseous lesions*.

I. OSSEOUS (BONE MARROW) LESIONS. In more than 95% of cases, multiple myeloma begins in the bone marrow. In the majority of cases, the disease involves multiple bones. By the time the diagnosis is made, most of the bone marrow is involved. Most commonly affected bones are those with red marrow i.e. skull, spine, ribs and pelvis, but later long bones of the limbs are also involved (Fig. 37.6). The lesions of myeloma secrete osteoclast-stimulating factor resulting in rarefaction of the bones. The lesions begin in the medullary cavity, erode the cancellous bone and ultimately cause destruction of the bony cortex. Radiographically, these lesions appear as punched out, rounded, 1-2 cm sized defects in the affected bone.

Grossly, the normal bone marrow is replaced by soft, gelatinous, reddish-grey tumours. The affected bone usually shows focal or diffuse osteoporosis.

Microscopically, the diagnosis of multiple myeloma can be usually established by examining bone marrow aspiration from an area of bony rarefaction. However, if the bone marrow aspiration yields dry tap or negative results, biopsy of radiologically abnormal or tender site is usually diagnostic. The following features characterise a case of myeloma:

- i) **Cellularity:** There is usually hypercellularity of the bone marrow.
- ii) **Myeloma cells:** Myeloma cells constitute 15-30% of the marrow cellularity. These cells may form clumps or sheets, or may be scattered among the normal haematopoietic cells. Myeloma cells may vary in size from small, differentiated cells resembling normal plasma cells to large, immature and

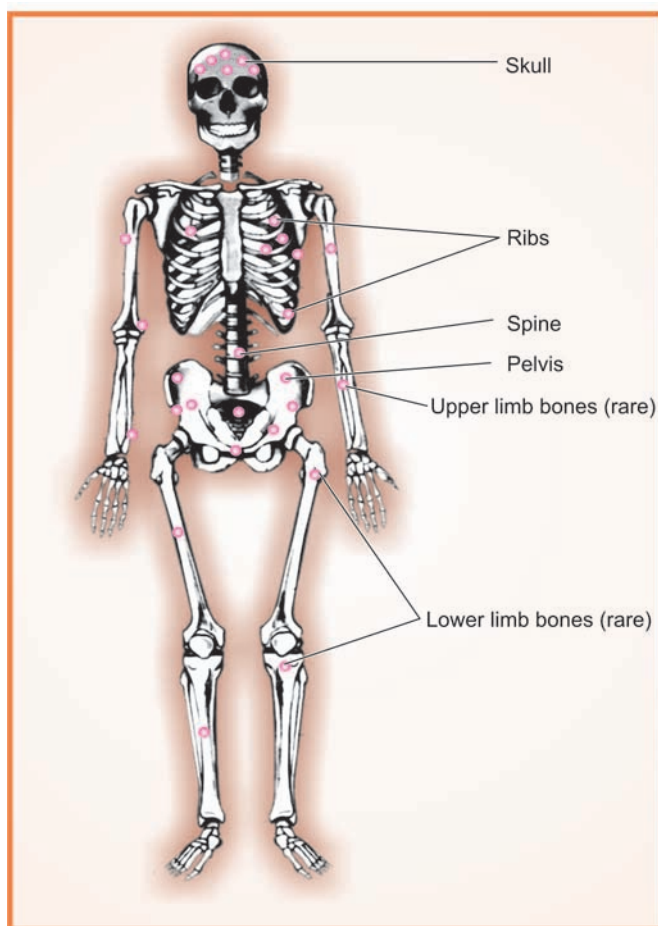


FIGURE 37.6

The major sites of lesions in multiple myeloma.

undifferentiated cells. Binucleate and multinucleate cells are sometimes present. The nucleus of myeloma cell is commonly eccentric similar to plasma cells but usually lacks the cart-wheel chromatin pattern seen in classical plasma cells. Nucleoli are frequently present. The cytoplasm of these cells is abundant and basophilic with perinuclear halo, vacuolisation and contains Russell bodies consisting of hyaline globules composed of synthesised immunoglobulin (Fig. 37.7).

In addition to multiple myeloma, *plasmacytosis* in the bone marrow can occur in some other disorders. These are: aplastic anaemia, rheumatoid arthritis, SLE, cirrhosis of liver, metastatic cancer and chronic inflammation. However, in all these conditions the plasma cells are mature and they do not exceed 10% of the total marrow cells.

II. EXTRAOSSEOUS LESIONS. Late in the course of disease, lesions at several extraosseous sites

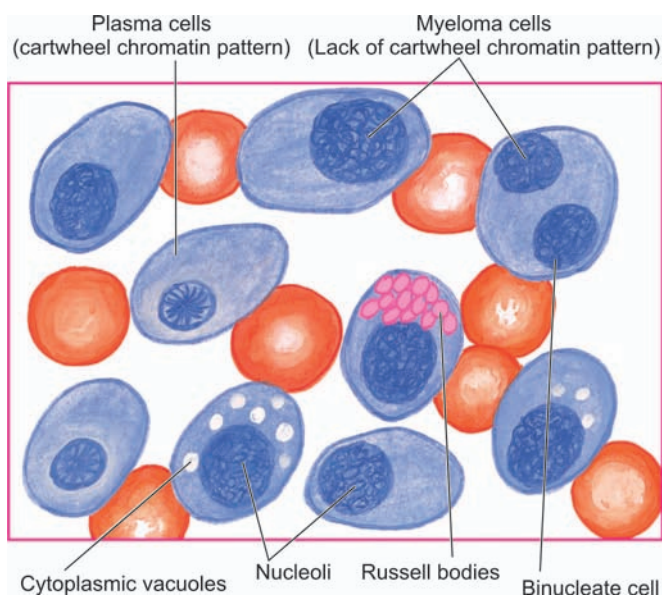


FIGURE 37.7

Bone marrow aspirate in myeloma showing numerous plasma cells, many with abnormal features.

become evident. Some of the commonly involved sites are as under:

- 1. Blood.** Approximately 50% of patients with multiple myeloma have a few atypical plasma cells in the blood. Other changes in the blood in myeloma are the presence of anaemia (usually normocytic normochromic type), marked red cell rouleaux formation due to hyperviscosity of blood, and an elevated ESR.
- 2. Myeloma kidney.** Renal involvement in myeloma called myeloma nephrosis occurs in many cases. The main mechanism of myeloma kidney is by filtration of light chain proteins (Bence Jones proteins) which are precipitated in the distal convoluted tubules in combination with Tamm-Horsfall proteins as tubular casts. The casts may be surrounded by some multinucleate giant-cells and a few inflammatory cells.
- 3. Myeloma neuropathy.** Infiltration of the nerve trunk roots by tumour cells produces nonspecific polyneuropathy. Pathologic fractures, particularly of the vertebrae may occur causing neurologic complications.
- 4. Systemic amyloidosis.** Systemic primary generalised amyloidosis (AL amyloid) may occur in 10% cases of multiple myeloma and involve multiple organs and systems.
- 5. Liver, spleen involvement.** Involvement of the liver and spleen by myeloma cells sufficient to cause

hepatomegaly, and splenomegaly occurs in a small percentage of cases.

Clinical Features

The clinical manifestations of myeloma result from the effects of infiltration of the bones and other organs by neoplastic plasma cells and from immunoglobulin synthesis. The principal clinical features are as under :

1. *Bone pain* is the most common symptom. The pain usually involves the back and ribs. Pathological fractures may occur causing persistent localised pain. Bone pain results from the proliferation of tumour cells in the marrow and activation of osteoclasts which destroy the bones. Myeloma cells release osteoclast-activating factor (OAF) which is mediated by several cytokines, mainly TNF and IL-1.
2. *Susceptibility to infections* is the next most common clinical feature. Particularly common are bacterial infections such as pneumonias and pyelonephritis. The increased susceptibility to infection is related mainly to hypogammaglobulinaemia, and partly to granulocyte dysfunction and neutropenia.
3. *Renal failure* occurs in about 25% of patients, while renal pathology occurs in 50% of cases. Causes of renal failure in myeloma are hypercalcaemia, glomerular deposits of amyloid, hyperuricaemia and infiltration of the kidney by myeloma cells.
4. *Anaemia* occurs in majority of patients of myeloma and is related to marrow replacement by the tumour cells (myelophthisis) and inhibition of haematopoiesis.
5. *Bleeding tendencies* may appear in some patients due to thrombocytopenia, deranged platelet function and interaction of the M component with coagulation factors.
6. *Neurologic symptoms* occur in a minority of patients and are explained by hyperviscosity, cryoglobulins and amyloid deposits.
7. *Hyperviscosity syndrome* owing to hyperglobulinaemia may produce headache, fatigue, visual disturbances and haemorrhages.
8. *Biochemical abnormalities*. These include:
 - i) hypercalcaemia due to destruction of bone;
 - ii) hyperuricaemia from necrosis of tumour mass and from uraemia related to renal failure; and
 - iii) along with other globulins, there is increased β -2 microglobulins in urine and serum (considered as a single most important predictor of survival).

Diagnosis

The diagnosis of myeloma is made by classic *triad* of features:

1. marrow plasmacytosis of more than 10%;
2. radiologic evidence of lytic bony lesions; and
3. demonstration of serum and/or urine M component.

There is rise in the total serum protein concentration due to *paraproteinaemia*. Paraproteins are abnormal immunoglobulins or their parts circulating in plasma and excreted in urine. The most common paraprotein is IgG seen in two-third cases of myeloma, IgA in one-third, and rarely there may be IgM or IgD paraproteins, or various combinations. Though the commonest cause of paraproteinaemias is multiple myeloma, certain other conditions may also produce serum paraproteins such as:

- Waldenström's macroglobulinaemia;
- Benign monoclonal gammopathy;
- B-cell lymphomas;
- CLL;
- Light chain disease;
- Heavy chain disease; and
- Cryoglobulinaemia.

As a result of paraproteinaemia, normal serum immunoglobulins (IgG, IgA and IgM) and albumin are depressed. The paraprotein usually appears as a single narrow homogeneous *M-band* on the serum electrophoresis, most commonly in the region of γ -globulin (Fig. 37.8). About two-third cases of myeloma excrete Bence Jones (light chain) proteins in the urine, consisting of either kappa or lambda light chains, alongwith presence of Bence Jones paraproteins in the serum.

Two variants of myeloma which do not fulfil the criteria of classical triad are *solitary bone plasmacytoma* and *extramedullary plasmacytoma*. Both these are associated with M component in about a third of cases and occur in young individuals. Solitary bone plasmacytoma is a lytic bony lesion without marrow plasmacytosis. Extramedullary plasmacytoma involves most commonly the submucosal lymphoid tissue of nasopharynx or paranasal sinuses. Both variants have better prognosis than the classic multiple myeloma. *Plasma cell granuloma*, on the other hand, is an inflammatory condition having admixture of other inflammatory cells with mature plasma cells.

Prognosis

Treatment of multiple myeloma consists of systemic chemotherapy in the form of alkylating agents and symptomatic supportive care. The median survival is 2 years after the diagnosis is made. The terminal phase is marked by the development of pancytopenia, severe anaemia and sepsis.

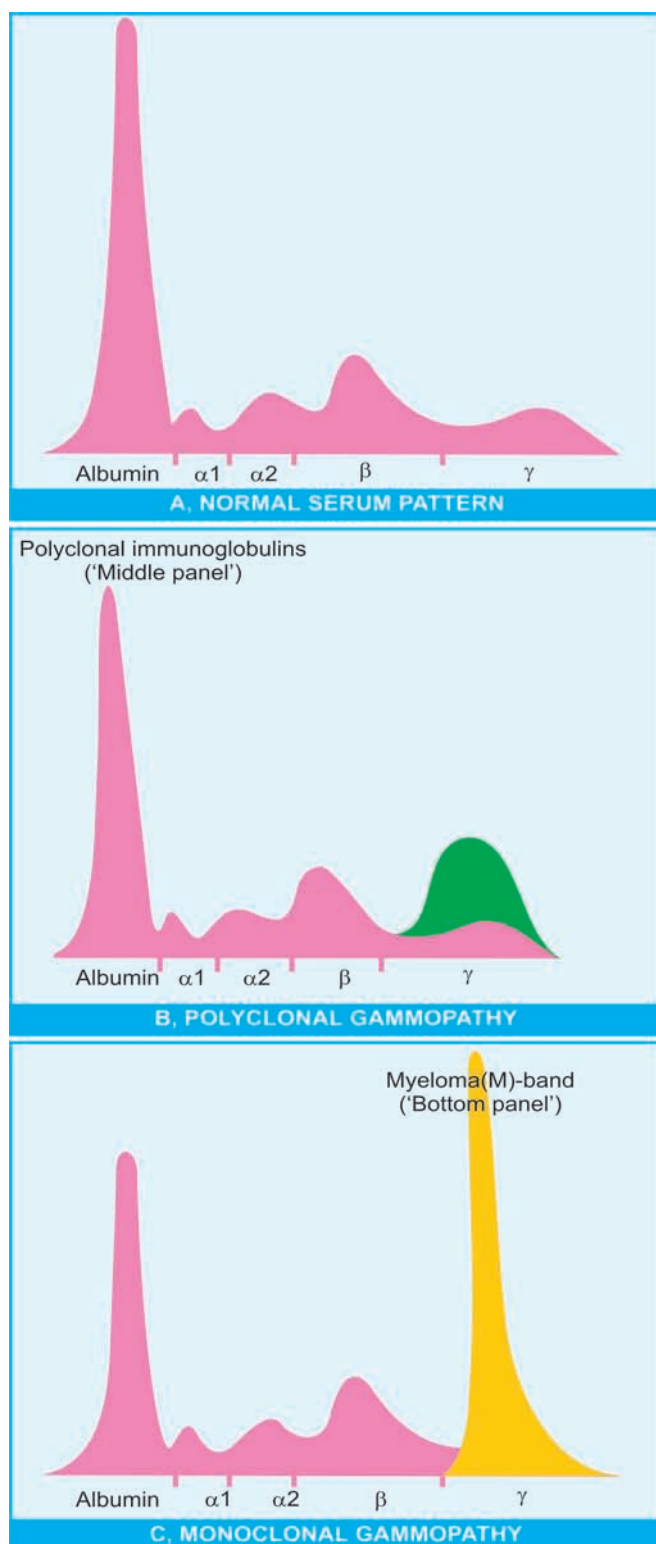


FIGURE 37.8

Serum electrophoresis showing normal serum pattern (A), as contrasted with that in polyclonal gammopathy (B) and in monoclonal gammopathy (C).

LANGERHANS' CELL HISTIOCYTOSIS

Langerhans' cell histiocytosis is a group of malignant proliferations of histiocytes and macrophages. Earlier, this group was referred to as histiocytosis-X and included three conditions: eosinophilic granuloma, Hand-Schüller-Christian disease and Letterer-Siwe syndrome. Now, it is known that:

- *firstly*, histiocytosis-X are not proliferations of unknown origin (X-for unknown) but proliferating cells are in fact Langerhans' cells of marrow origin. Langerhans' cells are normally present mainly in the epidermis but also in some other organs; and

- *secondly*, the three conditions included under histiocytosis-X are actually different expression of the same basic disorder. This has been made possible by demonstration of common antigens on these cells (positive for S-100 protein, CD1, CD 74 and HLA-DR) as well as by ultrastructural features of Langerhans' cell or Birbeck granules in the cytoplasm.

The three disorders included in the group are briefly considered below.

Eosinophilic Granuloma

Unifocal eosinophilic granuloma is more common (60%) than the multifocal variety which is often a component of Hand-Schüller-Christian disease (described below). Most of the patients are children and young adults, predominantly males. The condition commonly presents as a solitary osteolytic lesion in the femur, skull, vertebrae, ribs and pelvis. The diagnosis requires biopsy of the lytic bone lesion.

Microscopically, the lesion consists largely of closely-packed aggregates of macrophages admixed with variable number of eosinophils. The macrophages contain droplets of fat or a few granules of brown pigment indicative of phagocytic activity. A few multinucleate macrophages may also be seen. The cytoplasm of a few macrophages may contain rod-shaped inclusions called *histiocytosis-X bodies*.

Clinically, unifocal eosinophilic granuloma is a benign disorder. The bony lesion remains asymptomatic until the erosion of the bone causes pain or fracture. Spontaneous fibrosis or healing may occur in some cases, while others may require curettage or radiotherapy.

Hand-Schüller-Christian Disease

A triad of features consisting of *multifocal bony defects*, *diabetes insipidus* and *exophthalmos* is termed Hand-Schüller-Christian disease. The disease develops in children under 5 years of age. The multifocal lytic bony

lesions may develop at any site. Orbital lesion causes exophthalmos, while involvement of the hypothalamus causes diabetes insipidus. Multiple spherical lesions in the lungs are frequently present. Half the patients have involvement of the liver, spleen and lymph nodes.

Microscopically, the lesions are indistinguishable from those of unifocal eosinophilic granuloma.

Clinically, the affected children frequently have fever, skin lesions, recurrent pneumonitis and other infections. Though the condition is benign, it is more disabling than the unifocal eosinophilic granuloma. The lesions may resolve spontaneously or may require chemotherapy or radiation.

Letterer-Siwe Disease

Letterer-Siwe disease is an acute clinical syndrome of unknown etiology occurring in infants and children under three years of age. The disease is characterised by hepatosplenomegaly, lymphadenopathy, thrombocytopenia, anaemia and leucopenia. There is generalised hyperplasia of tissue macrophages in various organs.

Microscopically, the involved organs contain aggregates of macrophages which are pleomorphic and show nuclear atypia. The cytoplasm of these cells contains vacuoles and rod-shaped *histiocytosis-X* bodies.

Clinically, the child has acute symptoms of fever, skin rash, loss of weight, anaemia, bleeding disorders and enlargement of lymph nodes, liver and spleen. Cystic bony lesions may be apparent in the skull, pelvis and long bones. Intense chemotherapy helps to control Letterer-Siwe disease but intercurrent infections result in fatal outcome in many cases. The condition is currently regarded as an unusual form of malignant lymphoma.

SPLEEN

NORMAL STRUCTURE

The spleen is the largest lymphoid organ of the body. Under normal conditions, the average weight of the spleen is about 150 gm in the adult. Normally, the organ lies well protected by the 9th, 10th and 11th ribs in the upper left quadrant. The surface of the spleen is covered by a layer of peritoneum underneath which the organ is ensheathed by a thin *capsule*. From the capsule extend connective tissue *trabeculae* into the pulp of the organ and serve as supportive network. Blood enters the spleen by the splenic artery which divides into branches that penetrate the spleen via trabeculae. From the trabe-

culae arise small branches called *central arterioles*. Blood in the central arterioles empties partly into splenic venules and from there into splenic vein, but largely into vascular sinuses of the red pulp and thence into the splenic venous system.

Grossly, the spleen consists of homogeneous, soft, dark red mass called the *red pulp* and long oval grey-white nodules called the *white pulp* (*malpighian bodies*).

Microscopically, the red pulp consists of a network of thin-walled venous sinuses and adjacent blood spaces. The blood spaces contain blood cells, lymphocytes and macrophages and appear to be arranged in cords called *splenic cords* or *cords of Billroth*. The white pulp is made up of lymphocytes surrounding an eccentrically placed arteriole. The periarteriole lymphocytes are mainly T-cells, while at other places the lymphocytes have a germinal centre composed principally of B-cells surrounded by densely packed lymphocytes.

The spleen is a lymphoreticular organ that performs at least the following four *functions*:

1. Like other lymphoid tissues, it is an organ of the immune system where B and T lymphocytes multiply and help in *immune responses*.
2. The spleen plays an active role in *sequestering* and removing normal and abnormal blood cells.
3. The vasculature of the spleen plays a role in *regulating portal blood flow*.
4. Under pathologic conditions, the spleen may become the site of *extramedullary haematopoiesis*.

The spleen is rarely the primary site of disease. Being the largest lymphoreticular organ, it is involved secondarily in a wide variety of systemic disorders which manifest most commonly as splenic enlargement (splenomegaly) described below. A few other systemic involvements such as splenic infarcts and chronic venous congestion (CVC) of spleen have already been considered in Chapter 19 (page 199).

SPLENOMEGALY

Enlargement of the spleen termed splenomegaly, occurs in a wide variety of disorders which increase the cellularity and vascularity of the organ. Many of the causes are exaggerated forms of normal splenic function. Splenic enlargement may occur as a result of one of the following pathophysiologic mechanisms:

- I. Infections
- II. Disordered immunoregulation
- III. Altered splenic blood flow

- IV. Lymphohaematogenous malignancies
- V. Diseases with abnormal erythrocytes
- VI. Storage diseases
- VII. Miscellaneous causes.

Based on these mechanisms, an abbreviated list of causes of splenomegaly is given in Table 37.5. Most of these conditions have been discussed elsewhere.

The **degree of splenomegaly** varies with the disease entity:

- **Mild enlargement (upto 5 cm)** occurs in CVC of spleen in CHF, acute malaria, typhoid fever, bacterial endocarditis, SLE, rheumatoid arthritis and thalassaemia minor.
- **Moderate enlargement (upto umbilicus)** occurs in hepatitis, cirrhosis, lymphomas, infectious mononucleosis, haemolytic anaemia, splenic abscesses and amyloidosis.
- **Massive enlargement (below umbilicus)** occurs in CML, myeloid metaplasia with myelofibrosis, storage diseases, thalassaemia major, chronic malaria, leishmaniasis and portal vein obstruction.

Mild to moderate splenomegaly is usually symptomless, while a massively enlarged spleen may cause dragging sensation in the left hypochondrium. Spleen becomes palpable only when it is enlarged.

Grossly, an enlarged spleen is heavy and firm. The capsule is tense and thickened. The sectioned surface of the organ is firm with prominent trabeculae.

Microscopically, there is dilatation of sinusoids with prominence of splenic cords. The white pulp is atrophic while the trabeculae are thickened. Long-standing congestion may produce haemorrhages and *Gamna-Gandy bodies* resulting in *fibrocongestive splenomegaly*, also called *Banti's spleen* (Fig. 37.9) (COLOUR PLATE III: CL 9).

HYPERSPLENISM

The term hypersplenism is used for conditions which cause excessive removal of erythrocytes, granulocytes or platelets from the circulation. The mechanism for excessive removal could be due to increased sequestration of cells in the spleen by altered splenic blood flow or by production of antibodies against respective blood cells. The criteria for hypersplenism are as under:

1. Splenomegaly.
2. Splenic destruction of one or more of the cell types in the peripheral blood causing anaemia, leucopenia, thrombocytopenia, or pancytopenia.
3. Bone marrow cellularity is normal or hyperplastic.

TABLE 37.5: Causes of Splenomegaly.

I. INFECTIONS

1. Malaria
2. Leishmaniasis
3. Typhoid
4. Infectious mononucleosis
5. Bacterial septicaemia
6. Bacterial endocarditis
7. Tuberculosis
8. Syphilis
9. Viral hepatitis
10. AIDS

II. DISORDERS OF IMMUNOREGULATION

1. Rheumatoid arthritis
2. SLE
3. Immune haemolytic anaemias
4. Immune thrombocytopenias
5. Immune neutropenias

III. ALTERED SPLENIC BLOOD FLOW

1. Cirrhosis of liver
2. Portal vein obstruction
3. Splenic vein obstruction
4. Congestive heart failure

IV. LYMPHO-HAEMATOGENOUS MALIGNANCIES

1. Hodgkin's disease
2. Non-Hodgkin's lymphomas
3. Multiple myeloma
4. Leukaemias
5. Myeloproliferative disorders (e.g. CML, polycythaemia vera, myeloid metaplasia with myelofibrosis)

V. DISEASES WITH ABNORMAL ERYTHROCYTES

1. Thalassaemias
2. Spherocytosis
3. Sickle cell disease
4. Ovalocytosis

VI. STORAGE DISEASES

1. Gaucher's disease
2. Niemann-Pick's disease

VII. MISCELLANEOUS

1. Amyloidosis
2. Primary and metastatic splenic tumours
3. Idiopathic splenomegaly

4. Splenectomy is followed by improvement in the severity of blood cytopenia.

EFFECTS OF SPLENECTOMY

In view of the prominent role of normal spleen in sequestration of blood cells, splenectomy in a normal individual is followed by significant haematologic

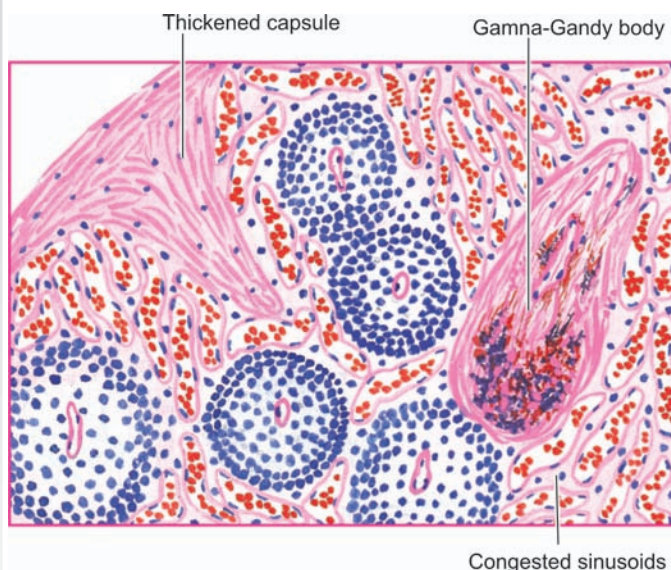


FIGURE 37.9

Microscopic characteristics in fibrocongestive splenomegaly. There is dilatation and congestion of splenic sinusoids with areas of haemorrhages and presence of a Gamna-Gandy body. The trabeculae are prominent while the white pulp is atrophic.

alterations. Induction of similar haematologic effects is made use in the treatment of certain pathologic conditions. For example, in autoimmune haemolytic anaemia or thrombocytopenia, the respective blood cell counts are increased following splenectomy. The blood changes following splenectomy are as under:

- 1. Red cells:** There is appearance of target cells in the blood film. Howell-Jolly bodies are present in the red cells as they are no longer cleared by the spleen. Osmotic fragility test shows increased resistance to haemolysis. There may be appearance of normoblasts.
- 2. White cells:** There is leucocytosis reaching its peak in 1-2 days after splenectomy. There is shift-to-left of the myeloid cells with appearance of some myelocytes.

- 3. Platelets:** Within hours after splenectomy, there is rise in platelet count upto 3-4 times normal.

SPLENIC RUPTURE

The most common cause of splenic rupture or laceration is blunt trauma. The trauma may be direct or indirect. Non-traumatic or spontaneous rupture occurs in an enlarged spleen but almost never in a normal spleen. In acute infections, the spleen can enlarge rapidly to 2 to 3 times its normal size causing acute splenic enlargement termed *acute splenic tumour* e.g. in pneumonias, septicaemia, acute endocarditis etc. Some of the other common causes of spontaneous splenic rupture are splenomegaly due to chronic malaria, infectious mononucleosis, typhoid fever, splenic abscess, thalassaemia and leukaemias.

Rupture of spleen is an acute surgical emergency due to rapid blood loss and haemoperitoneum. Sometimes fragments of splenic tissue are autotransplanted within the peritoneal cavity and grow into tiny spleens there (*splenosis*).

TUMOURS

■ **Primary tumours** of the spleen are extremely rare. The only notable benign tumours are haemangiomas and lymphangioma, while examples of primary malignant neoplasms are Hodgkin's disease and non-Hodgkin's lymphomas. Non-haematopoietic tumours of the spleen are rare.

■ **Secondary tumours** occur late in the course of disease and represent haematogenous dissemination of the malignant tumour. Splenic metastases appear as multiple nodules. The most frequent primary sites include: lung, breast, prostate, colon and stomach. Rarely, direct extension from an adjacent malignant neoplasm may occur.



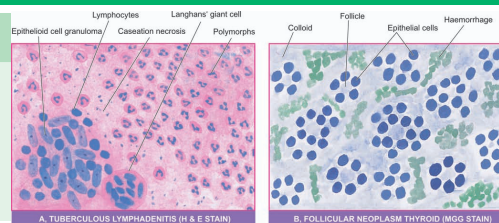
Miscellaneous

SECTION CONTENTS

CHAPTER 38: Basic Diagnostic Cytopathology

CHAPTER 39: Function Tests

CHAPTER 40: Normal Values



38

Basic Diagnostic Cytopathology

INTRODUCTION

As mentioned in the beginning of this book, diagnostic surgical pathology developed as a branch around the turn of 19th Century. Its basis was application of knowledge of morphological details of cells for diagnosis of disease in biopsy material. This generated interest by workers towards obtaining cellular material by non-biopsy techniques to arrive at the diagnosis. As a result, cytopathologic diagnosis was initially introduced purely as *Exfoliative Cytology* in the 1920s by Dr. George N. Papanicolaou. Subsequently, *Aspiration Biopsy* was introduced in the 1930s, and evolved over the next three decades (mainly in Europe) to become the mainstay branch of diagnostic cytology known as *Interventional Cytology*.

In general, diagnostic cytology pertains to the interpretation of cells from the human body that either exfoliate (desquamate) spontaneously from epithelial surfaces, or are obtained from various organs/tissues by different clinical procedures. While histopathologic diagnosis is based on interpretation of changes in tissue architecture, the cytopathologic diagnosis rests upon alterations in morphology observed in single cells or groups of cells.

APPLICATIONS OF DIAGNOSTIC CYTOLOGY

The major applications of cytodiagnostic techniques are as under:

1. Diagnosis and management of cancer. In the field of oncology, establishing a 'tissue diagnosis' (i.e. patho-

logic diagnosis based on microscopic evidence of malignancy) is an essential pre-requisite for proper management of a cancer patient.

i) Cytodiagnosis in its traditional role is a *valuable adjunct* to histopathology for establishing the vital tissue diagnosis e.g. diagnosis of lymphomas where imprint smears prove valuable, and in some respects superior to histopathology in typing the lymphoma.

ii) Cytologic techniques also provide a *preliminary diagnosis* of cancer for later confirmation by histopathology e.g. detection of ovarian cancer cells in ascitic fluid.

iii) In some fields, cytodiagnosis has replaced histopathology as the *primary source/method* of establishing a tissue diagnosis e.g. in breast cancer, where positive cytologic report is considered sufficient for planning the management, obviating the need for diagnostic surgical biopsy.

iv) Cytodiagnosis has a major role in the detection and diagnosis of clinically silent *early cancer* e.g. carcinoma *in situ* of the uterine cervix.

v) In the management of cancer, cytodiagnosis may help in assessing *response to therapy* e.g. cervical smears for response to radiotherapy in carcinoma cervix, and urinary cytology for response to chemotherapy in carcinoma of the urinary bladder.

vi) In the *follow-up* of previously diagnosed/known cases of cancer, it is of particular value in detecting dissemination (metastatic spread) or recurrence of tumour.

2. Identification of benign neoplasms. This application is derived from its ability to distinguish between

benign and malignant neoplasms e.g. fibroadenoma of the breast *versus* carcinoma.

3. Intraoperative pathologic diagnosis. In this role, cytodiagnosis complements histopathologic diagnosis e.g. imprint smears alongwith frozen section for breast lumps.

4. Diagnosis of non-neoplastic/inflammatory conditions. Cytodiagnosis allows recognition of specific conditions which do not routinely require surgical intervention e.g. Hashimoto's thyroiditis.

5. Diagnosis of specific infections. A variety of bacterial, viral, protozoal and fungal infections can be identified by cytologic methods e.g. tubercle bacilli in lymph node aspirates, herpetic inclusions and *Trichomonas* in cervico-vaginal smears, fungal hyphae in touch preparations from draining sinuses.

6. Cytogenetics. Cytodiagnostic techniques can be employed for chromosomal studies including leucocyte and tissue cultures and for demonstrating sex-chromatin e.g. buccal smear for Barr body.

7. Assessment of hormonal status in women. Vaginal smears accurately reflect changes in female sex hormonal levels e.g. to confirm the onset of menopause.

8. Identification of tissues/cells. For example, identification of spermatogenic elements in aspirates from the testes in cases of male infertility.

For cytomorphological *recognition of cancer*, *nuclear characteristics* given in Table 38.1 are used to determine the presence or absence of malignancy. *Cytoplasmic characteristics* help in *typing the malignancy* e.g. keratinisation in squamous cell carcinoma, mucin droplets in adenocarcinoma, melanin pigment in melanomas.

BRANCHES OF DIAGNOSTIC CYTOLOGY

Two branches of diagnostic cytology are currently recognised: exfoliative and interventional.

EXFOLIATIVE CYTOLOGY. This is the older branch that essentially involves the study of cells spontaneously shed off (as a result of continuous growth of epithelial linings) from epithelial surfaces into body cavities or

TABLE 38.1: Nuclear Criteria of Malignancy.

1. <i>Nuclear size</i>	: Usually larger than benign nuclei; variation in size (anisonucleosis) more significant.
2. <i>Nucleus-cytoplasmic (N:C) ratio</i>	: Increased.
3. <i>Nuclear shape</i>	: Moderate to marked variation.
4. <i>Nuclear membrane</i>	: Irregular thickening, angulation and indentations.
5. <i>Nuclear chromatin</i>	: Hyperchromatic (less significant), uneven distribution, coarse irregular angulated chromatin clumping, parachromatin clearing (more significant).
6. <i>Nucleoli</i>	: Increased size and number less significant; irregular angular outlines more significant.
7. <i>Number of nuclei</i>	: Multinucleation unreliable; nuclear character more important.
8. <i>Mitoses</i>	: Increased mitoses unreliable; abnormal mitoses significant.

body fluids. Exfoliative cytology is facilitated by the fact that the rate of exfoliation is enhanced in disease-states thereby yielding a larger number of cells for study. In addition, cells for study may also be obtained by scraping, brushing, or washing various mucosal surfaces (abrasive cytology).

INTERVENTIONAL CYTOLOGY. This is the branch in which samples are obtained by clinical procedures or surgical intervention. It is dominated by, and is virtually synonymous with, Fine Needle Aspiration Cytology (FNAC) which is also known as Aspiration Biopsy Cytology (ABC). In interventional cytology, the cytopathologist often performs the clinical procedure himself and interacts with the patient in contrast to exfoliative cytology where the samples are prepared/forwarded by the clinician and the cytopathologist interprets them in isolation.

Contrasting features and differences between exfoliative and interventional cytology are presented in Table 38.2.

TABLE 38.2: Differences between Exfoliative and Interventional Cytology.

FEATURE	EXFOLIATIVE CYTOLOGY	INTERVENTIONAL CYTOLOGY
1. <i>Cell samples</i>	Exfoliated from epithelial surfaces	Obtained by intervention/aspiration
2. <i>Smears</i>	Require screening to locate suitable cells for study	Abundance of cells for study in most smears
3. <i>Diagnostic basis</i>	Individual cell morphology	Cell patterns and morphology of groups of cells
4. <i>Morphologic criteria of diagnostic significance</i>	Nuclear characteristics most important	Nuclear characteristics important; cytoplasmic character and background equally significant

EXFOLIATIVE CYTOLOGY

Type of samples that can be obtained from different organ systems for exfoliative cytodagnosis are listed in Table 38.3. Cytology of female genital tract and buccal smears for sex chromatin are briefly discussed below.

PAP SMEARS

Samples (smears) from the female genital tract have traditionally been known as 'Pap smears'. These smears may be prepared by different methods depending upon the purpose for which they are intended:

- i) *Lateral vaginal smears (LVS)* obtained by scraping the upper third of the lateral walls of the vagina are ideal for cytohormonal assessment.
- ii) *Vaginal 'pool' or 'vault' smears* are obtained by scraping or aspirating material from the posterior fornix of the vagina and are recommended for detection of endometrial and ovarian cancer.
- iii) *Cervical smears* obtained by cotton swabs or Ayre's spatula from the portio of the cervix are ideal for detection of cervical carcinoma.
- iv) *Combined (Fast) smears* are a combination of vaginal pool and cervical scrapings. They offer the advantages of both and are recommended for routine population screening as they allow detection of up to 97% of cervi-

cal cancers and about 90% of endometrial cancers when properly prepared.

v) *Triple smears (cervical-vaginal-endocervical or CVE)* contain three distinct samples representing the ectocervix, vagina and endocervix on three separate areas of the same slide. These smears are also recommended for routine screening as they allow localisation of lesions but are difficult to prepare.

vi) *Endocervical and endometrial smears* may also be prepared by aspirating the contents of the endocervical canal and endometrial cavity respectively.

Normally, there are 4 types of squamous epithelial cells in the cervical smear: *superficial, intermediate, parabasal and basal cells*. Morphological features of these cells are summed up in Table 38.4 and illustrated in Fig. 38.1.

APPLICATIONS OF PAP SMEAR

Cytohormonal Evaluation

Assessment of hormonal status is best carried out from lateral vaginal smears although vaginal 'pool' or fast smears may also be used. Ideally, at least 3 smears obtained on alternate days should be scrutinised and cytologic indices determined for each smear for accurate assessment.

Abnormal Combined Smears

In order to evolve a system acceptable to clinicians and cytopathologists, National Cancer Institute Workshop in 1988 developed *The Bethesda System* for uniformity in

TABLE 38.3: Types of Samples for Exfoliative Cytology: Obtainable from Different Organ Systems/Body Sites.

1. <i>Female genital tract</i>	— Lateral vaginal smears (LVS) — Vaginal 'pool' smears — Cervical smears — Combined (fast) smears — Triple smears (CVE) — Endocervical/Endometrial aspiration
2. <i>Respiratory tract</i>	— Sputum — Bronchial washings/brushing/bronchio-alveolar lavage (BAL)
3. <i>Gastrointestinal tract</i>	— Endoscopic lavage/brushing
4. <i>Urinary tract</i>	— Urinary sediment — Bladder washings — Retrograde catheterisation — Prostatic massage (secretions)
5. <i>Body fluids</i>	— Effusions — Fluids of small volume i. Cerebrospinal fluid (CSF) ii. Synovial fluid iii. Amniotic fluid iv. Hydrocele fluid v. Seminal fluid (semen) vi. Nipple discharge
6. <i>Other samples</i>	— Buccal smears (for sex chromatin)

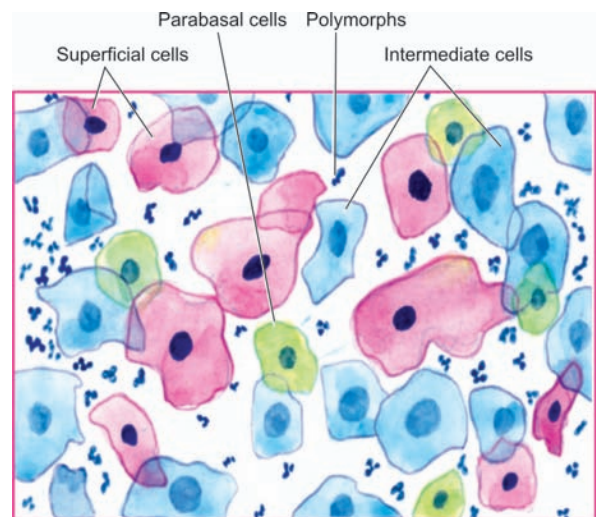


FIGURE 38.1

Various types of cells seen in Pap smear.

TABLE 38.4: Squamous Epithelial Cells Found in Normal Combined (Fast) Smears.

CELL TYPE	SIZE	NUCLEI	CYTOPLASM	MORPHOLOGY
<i>Superficial</i>	30-60 μm	< 6 μm dark, pyknotic	Polyhedral, thin, broad, acidophilic or cyanophilic with keratohyaline granules.	
<i>Intermediate</i>	20-40 μm	6-9 μm vesicular	Polyhedral or elongated, thin, cyanophilic with folded edges.	
<i>Parabasal</i>	15-25 μm	6-11 μm vesicular	Round to oval, thick, well-defined, basophilic with occasional small vacuoles.	
<i>Basal</i>	13-20 μm	Large, (> one-half of cell volume), hyperchromatic, may have small nucleoli	Round to oval, deeply basophilic.	

evaluation as well as limitations of reporting in cervico-vaginal cytopathology; this was subsequently modified in 1991 and further updated in 2001. Briefly, it has three basic components:

- 1. Specimen adequacy:** It is an important component of quality assurance and provides feedback regarding sampling technique. It implies properly labelled, adequately fixed smears having sufficient number of evenly spread, well preserved cells on microscopic evaluation.
- 2. General categorisation:** It includes categorising the smear in one of the three broad categories: within normal limits, benign cellular changes, and epithelial cell abnormalities.
- 3. Descriptive diagnosis:** Final aspect of the Bethesda system includes detailed description of the benign cellular changes or epithelial cell abnormalities in the smear.

Based on it, the cellular changes in cervical smears are discussed below under 2 headings: benign (non-neoplastic) cellular changes and epithelial cell abnormalities.

BENIGN (NON-NEOPLASTIC) CELLULAR CHANGES:

i) NON-SPECIFIC INFLAMMATORY CHANGES. Inflammatory changes not associated with any specific infection/identifiable infectious agent (i.e. non-specific inflammation) are commonly seen in smears of the female genital tract.

ii) SPECIFIC INFLAMMATORY CHANGES. Specific inflammatory changes may be associated with a variety of infectious agents, the common among which are listed in Table 38.5.

EPITHELIAL CELL ABNORMALITIES (NEOPLASTIC):

Carcinoma of the uterine cervix still ranks high in the list as the most frequent cancer in third world countries and is the leading cause of morbidity and mortality. The vast majority of cervical cancers are of the squamous or epidermoid type, and the diagnosis of squamous cell carcinoma of the cervix may be considered the single most important role of exfoliative cytology.

Neoplastic epithelial changes are described below under 2 headings: squamous cell abnormalities and glandular cell abnormalities.

1. SQUAMOUS CELL ABNORMALITIES: The fully-developed invasive squamous cell carcinoma of the uterine cervix is preceded by a pre-invasive intraepithelial neoplastic process that is recognisable on histologic and cytologic examination.

Morphogenesis and nomenclature. The earliest recognisable change is *hyperplasia* of basal or reserve cells which normally constitute a single layer at the deepest

TABLE 38.5: Common Infections Detectable on Pap Smears.

Bacterial agents:

Neisseria gonorrhoeae
Gardnerella vaginalis
Mycobacterium tuberculosis

Viral agents:

Herpes simplex virus (HSV)
Human papilloma virus (HPV)

Fungal agents:

Candida albicans
Torulopsis glabrata

Parasitic agents:

Trichomonas vaginalis
Entamoeba histolytica

part of the epithelium. The proliferating reserve cells next develop certain atypical features i.e. hyperchromasia and increased nuclear size. The continuing proliferation of these atypical cells with loss of polarity, a concomitant increase in mitotic activity, and progressive involvement of more and more layers of the epithelium is known as *dysplasia* (disordered growth). When dysplasia involves the full thickness of the epithelium and the lesion morphologically resembles squamous cell carcinoma without invasion of underlying stroma, it is termed *carcinoma in situ* (CIS). CIS further evolves through the stage of *microinvasive carcinoma* (with depth of stromal invasion not exceeding 3 mm) into full-blown *invasive squamous cell carcinoma* (Fig. 38.2).

Previously depending on the degree of epithelial involvement, three grades of dysplasia were recognised:

mild, moderate and severe. As the stages of dysplasia and CIS represented a continuous spectrum of lesions seen in the precancerous state, they were collectively termed 'cervical intraepithelial neoplasia' (CIN) and categorised as under:

CIN I	Mild dysplasia	Primitive (atypical) cells proliferating in lower third of epithelium.
CIN II	Moderate dysplasia	Involvement up to middle-third of epithelium.
CIN III	Severe dysplasia Carcinoma <i>in situ</i> (CIS)	Involvement of upper-third of epithelium. Involvement of full thickness of epithelium.

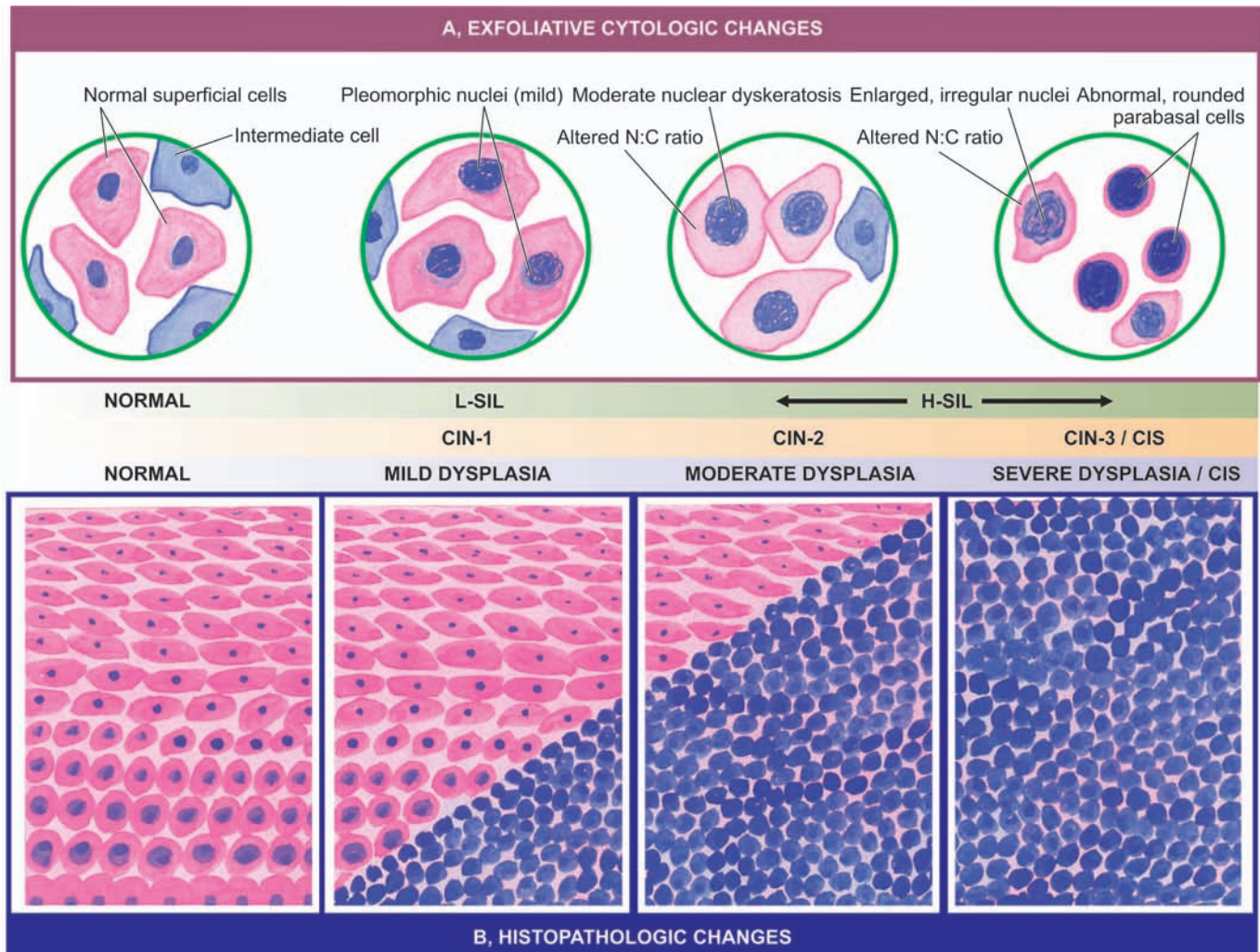


FIGURE 38.2

Cervical intraepithelial neoplasia (CIN) and squamous intraepithelial lesions (SIL). A, schematic representation of histologic changes (below). The grades of CIN-1 or mild dysplasia (L-SIL), CIN-2 (moderate dysplasia) and CIN-3 (severe dysplasia and carcinoma *in situ*) (together grouped as H-SIL) show progressive increase in the number of abnormal cells parallel to the increasing severity of grades. B, exfoliative cytologic studies in various grades of cellular changes (above).

Presently, the *Bethesda system* divides squamous cell abnormalities into four categories:

- *Atypical squamous cells of undetermined significance* (ASCUS) which represents cellular changes falling short of intraepithelial lesion.
- *Low-grade squamous intraepithelial lesion (L-SIL)* that includes CIN-I and cellular changes associated with HPV infection.
- *High-grade squamous intraepithelial lesion (H-SIL)* includes CIN grade II and III as well as CIS (**COLOUR PLATE VI: CL 22**).
- Squamous cell carcinoma.

2. GLANDULAR CELL ABNORMALITIES. The Bethesda system categorises glandular abnormalities as under:

- *Atypical glandular cells of undetermined significance* (AGUS) which represent nuclear atypia of endocervical and endometrial cells exceeding reparative changes.
- *Endocervical and endometrial adenocarcinoma*, both of which can be detected from Pap smears.
- Cells from *extrauterine cancers* may also be present in Pap smears, the majority originating from the ovaries. Clues to the extrauterine origin of cancer cells include: the absence of a tumour diathesis, arrangement of cancer cells in glandular and papillary configuration (accompanied by psammoma bodies in some instances), and absence of dysplastic changes in coexistent cervico-endometrial cells.

BUCCAL SMEARS FOR SEX CHROMATIN BODIES

Sex-specific chromatin bodies are observed in interphase nuclei and comprise the Barr body (X chromatin) and the fluorescent or F body (Y chromatin). The number of Barr bodies observed in interphase nuclei is one less than the number of X chromosome ($n-1$), whereas the number of F bodies observed is equal to the number of Y chromosomes present (n). In effect, the Barr body is specific for females and the F body for males.

1. DEMONSTRATION OF THE BARR BODY (X CHROMATIN). In buccal smears, the Barr body appears as a plano-convex mass about $1\ \mu\text{m}$ in diameter applied to the inner surface of the nuclear membrane. The interphase nuclei of one hundred intermediate squamous epithelial cells are scrutinised. In normal females, Barr bodies are present in at least 20% of nuclei. Males have a Barr body count of less than 2%. Vaginal smears may also be used for Barr body counts.

2. DEMONSTRATION OF THE F BODY (Y CHROMATIN). Demonstration of the F body requires fluorescent staining in contrast to the Barr body which may be observed on routine Papanicolaou-stained smears. On staining buccal smears with quinacrine mustard, the intensely fluorescent F body is observed in about 60% of interphase nuclei in males and in less than 8% of nuclei in females. The F body is also demonstrable in the nuclei of lymphocytes in a peripheral blood film stained with quinacrine mustard.

3. COUNTING OF DRUM-STICK APPENDAGE. The presence and frequency of drum-stick appendages attached to nuclei of polymorphonuclear leucocytes on a peripheral blood film may also be used for determining sex. At least 500 neutrophilic leucocytes are scrutinised in a Romanowsky-stained blood film. Genetic females show drumsticks in 3-6% of neutrophils. In males, the frequency of drumsticks is less than 0.3%.

INTERVENTIONAL CYTOLOGY

In interventional cytology, samples are obtained by aspiration or surgical biopsy. This branch includes FNAC, imprint cytology, crush smear cytology and biopsy sediment cytology.

I. FINE NEEDLE ASPIRATION CYTOLOGY

Interventional cytology is virtually synonymous with Fine Needle Aspiration Cytology or FNAC. The technique has gained wide acceptance only over the past three decades and is increasingly being used to sample a wide variety of body tissues. Almost all organ systems are accessible to the fine needle and the versatility of the technique has enormously increased the scope of diagnostic cytology.

Applications of FNAC

In routine practice, FNAC is most often used for diagnosis of palpable mass lesions. Palpable lesions commonly sampled are: breast masses, enlarged lymph nodes (Fig. 38.3,A) (**COLOUR PLATE XII: CL 48**), enlarged thyroid (Fig. 38.3, B) and superficial soft tissue masses. The salivary glands, palpable abdominal lesions and the testicles are also frequently sampled for FNAC. Other sites/lesions accessible to FNAC are: the prostate, pelvic organs, bone and joint spaces, lungs, retroperitoneum and orbit.

Advantages of FNAC

i) FNAC is an office/OPD procedure and *no hospitalisation* is required, while surgical biopsies are obtained in the operation theatre and hospitalisation is often required.

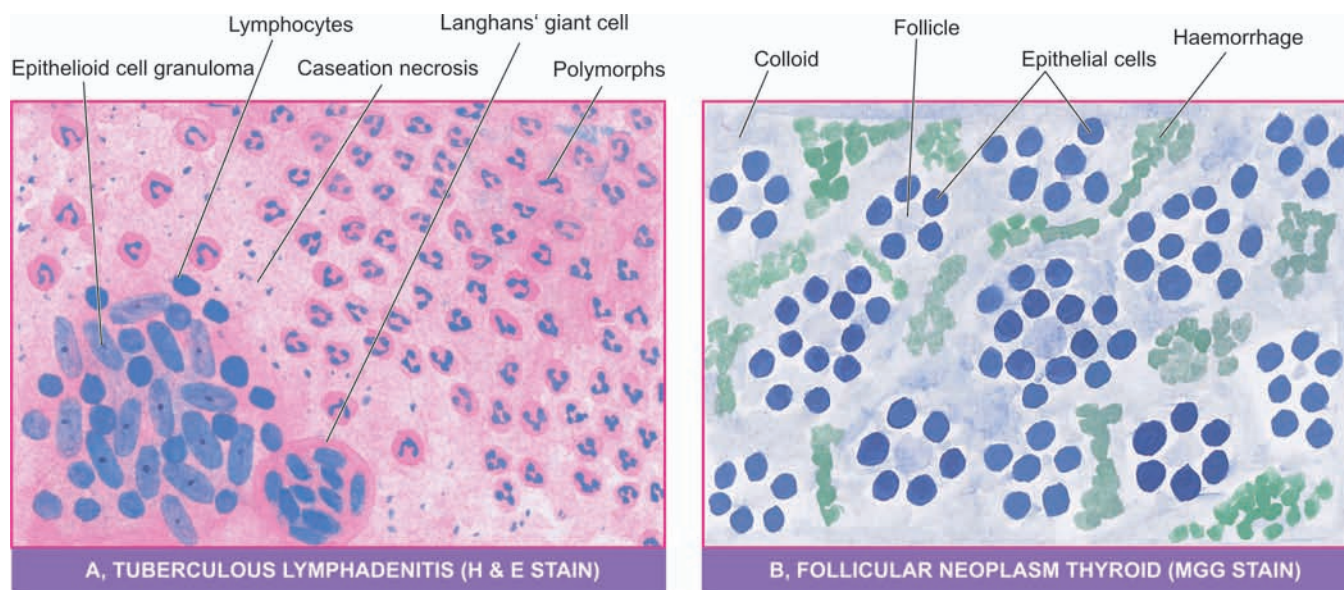


FIGURE 38.3

Microscopic appearance of FNA in tuberculosis of lymph node (A) and in follicular neoplasm of thyroid (B).

ii) *No anaesthesia* is required (except in specific circumstances), while surgical biopsy is performed under local or general anaesthesia.

iii) The procedure is *quick, safe and painless*.

iv) *Multiple attempts* (or repeating the procedure) are possible without inconvenience, whereas repeating a surgical biopsy is uncomfortable and inconvenient for the patient.

v) Results are obtained *rapidly* with reports being available in a matter of hours (2 to 24 hours depending on the urgency), while histopathological reports are available after a longer duration (2 to 4 days on account of time required for processing and sectioning of tissues).

vi) It is a low-cost procedure which is *cost-effective*.

vii) As the cytopathologist performs the procedure himself, he gains *first-hand knowledge* of the clinical findings which facilitates interpretation of slides and enhances diagnostic accuracy.

General Procedure for FNAC

Materials for FNAC

For performing FNAC, a syringe with a well-fitting needle, some microscopic glass slides and a suitable fixative are the only material required in most instances (Fig. 38.4).

NEEDLES. Fine needles range from 25 to 20 gauge (0.6 mm to 0.9 mm outer diameter). The standard 21

gauge disposable needle of 38 mm length is suitable for routine transcutaneous FNAC of palpable masses; 25 or 24 gauge disposable needles of 25 mm length are used for lymph nodes and in children. Larger needles, 80 to 160 mm in length, are required for sampling the lung and abdominal viscera; 22 to 20 gauge Chiba spinal puncture needles may be profitably employed for this purpose. Needles of up to 200 mm length are used for transrectal and transvaginal FNAC of the prostate and ovary respectively. Aspiration of bony lesions may require 18 gauge (1 mm outer diameter) needles although superficial lytic lesions are adequately sampled with a 21 gauge needle.

SYRINGES. Syringes of 10 to 20 ml capacity are suitable. Syringe holders (such as the Franzen handle, Fig. 38.4,B) permit a single-hand grip during aspiration, employing disposable syringes. For needles with metal hubs (e.g. spinal puncture needles), disposable syringes should be used to ensure a proper fit between the needle hub and syringe nozzle.

GLASS SLIDES AND FIXATIVE. Four or six standard microscopic glass slides and a Coplin jar containing 95% ethyl alcohol (as a fixative) are the only other equipment required for routine FNAC.

Method of Aspiration of FNAC

Transcutaneous FNAC of palpable masses is routinely performed without anaesthesia as per the following

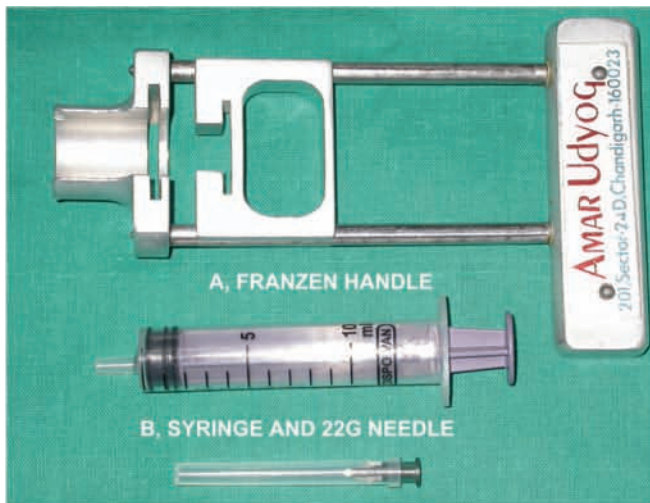


FIGURE 38.4

Equipments required for transcutaneous FNAC.



procedure (aspiration of sites/lesions requiring anaesthesia or special technique are discussed separately).

i) The patient is asked to *lie down* in a position that best exposes the target area.

ii) The target area is thoroughly *palpated* and the firmest portion of the lesion/mass delineated.

iii) The skin is *cleaned* with an alcohol pad.

iv) The mass is *fixed* by the palpating hand of the operator or by an assistant (gloves may be used for the protection of the operator and the assistant).

v) The needle is *inserted* into the target area. On reaching the lesion the plunger of the syringe is retracted and at least 10 ml of suction applied while moving the needle back and forth within the lesion; the direction (angle) of the needle may be changed to access different areas of the lesion (Fig. 38.5).

vi) Aspiration is *terminated* when aspirated material or blood becomes visible at the base/hub of the needle. For diagnostic purposes, cellular material contained within the needle is more than adequate; material drawn into the barrel of the syringe is not recovered since it is of no use for cytologic diagnosis.

vii) On completion of aspiration, suction is *released* and pressure within the syringe allowed to equalise before withdrawing the needle. Withdrawing the needle with negative pressure/suction results in blood being aspirated and cellular material being sucked into the barrel of the syringe, thus lost to interpretation.

viii) Following withdrawal of the needle from the lesion, *pressure* is applied to the site of puncture by the assistant/patient for 2 to 3 minutes in order to arrest bleeding and prevent haematoma formation.

ix) Aspirated material is *recovered* by detaching the needle from the syringe and filling the syringe with air; the syringe and needle are then reconnected and the aspirate expressed onto one end of a glass slide.

Preparation of Smears for FNAC

Preparation of smears is crucial to the success of FNAC. Poorly-prepared smears with distorted cellular

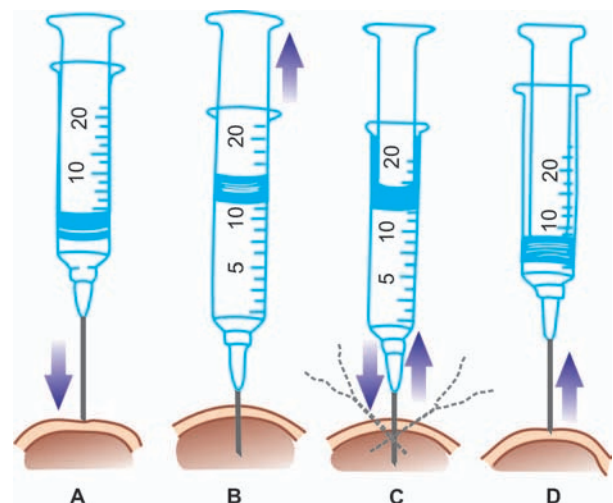


FIGURE 38.5

Procedure for FNAC of palpable masses. Needle is introduced into the mass (A). Plunger is retracted after needle enters the mass (B). Suction is maintained while needle is moved back and forth within the mass (C). Suction is released and plunger returned to original position before needle is withdrawn (D).

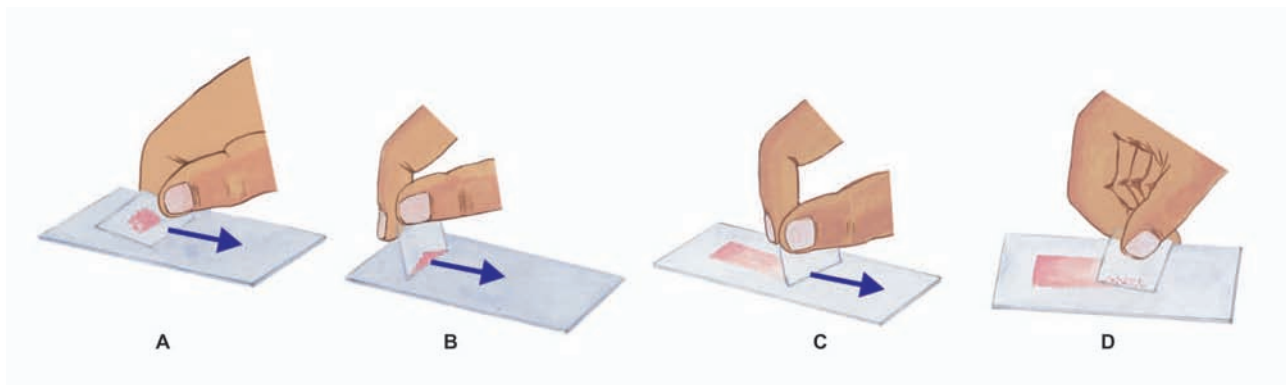


FIGURE 38.6

Preparation of smears. Semisolid aspirates are crush-smear by flat pressure with cover slip or glass slide (A). Fluid or blood droplet is collected along edge of spreader (B), and pulled as for peripheral blood films (C). Particles at the end of the smear are crush-smear (D).

morphology will frustrate the best efforts of the most competent cytopathologist, and often result in errors of interpretation or in failure to arrive at any specific diagnosis. The procedure consists of the following steps:

- i) Aspirates deposited on the slide are *inspected* with the naked eye.
- ii) Semisolid particulate aspirates are *crush-smear*ed by flat pressure with a glass slide or a thick coverslip (Fig. 38.6,A). Even and gentle pressure is required to avoid traumatising cells. Droplets of fluid or bloody material are gathered under the edge of the angled smearing slide/coverslip (Fig. 38.6,B) and pulled like a blood smear (Fig. 38.6,C). Particulate material, which collects along the edges and at the end of the smear, is then crush-smear-ed by flattening the smearing slide (Fig. 38.6,D).
- iii) Prepared smears are either *wet-fixed* or *air-dried*. Half the number of smears are immediately immersed in 95% ethanol, transported to the laboratory in the fixative, and used for Papanicolaou or haematoxylin and eosin (H&E) staining. The remaining smears are air-dried, wrapped in tissue paper for transport to the labo-

ratory, and stained by Romanowsky stains (Leishman's, May-Grünwald-Giemsa). Most cytopathologists use both wet-fixed and air-dried smears as the former provides excellent nuclear detail while the latter yields information about the cytoplasm and the background. The general properties of wet-fixed and air-dried smears are enumerated in Table 38.6.

Radiological Imaging Aids for FNAC

Non-palpable lesions require some form of localisation by radiological aids for FNAC to be carried out. *Plain X-ray films* are usually adequate for lesions within bones and for some lesions within the chest. FNAC of the chest may also be attempted under *image amplified fluoroscopy* which allows visualisation of needle placement on the television monitor. *Computerised tomography (CT scans)* can also be used for lesions within the chest and abdomen. The most versatile radiological aid is *ultrasonography (USG)* which allows direct visualisation of needle placement in real time and is free from radiation hazards. It is an extremely valuable aid for FNAC of thyroid nodules, soft tissue masses, intra-abdominal lesions and for intrathoracic lesions which abut

TABLE 38.6: General Properties of Wet-Fixed and Air-Dried Smears.		
FEATURE	WET-FIXED	AIR-DRIED
1. Staining	Papanicolaou, H&E	Leishman, May-Grünwald-Giemsa
2. Cell size	Comparable to tissue sections	Exaggerated
3. Nuclear detail	Excellent	Fair
4. Nucleoli	Well demonstrated	Not always discernible
5. Cytoplasmic details	Poorly demonstrated	Well demonstrated
6. Stromal components	Poorly demonstrated	Well demonstrated
7. Partially necrotic material	Single intact cells, well defined	Cell details poorly defined

the chest wall, but is of no help in deep intrathoracic lesions or in bony lesions.

Complications and Hazards of FNAC

FNAC is associated with relatively few complications. Possible hazards and more commonly encountered complications are as follows:

- 1. Haematomas.** Bleeding from the puncture site and haematoma formation are the commonest complications of the procedure, particularly in the breast and the thyroid. Firm finger pressure for 2 to 3 minutes immediately after the procedure greatly reduces the frequency of these complications.
- 2. Infection.** Introduction of infection is not a significant hazard; even trans-abdominal aspiration does not result in peritoneal contamination despite puncture of bowel walls. Transrectal aspiration in cases of acute prostatitis may, however, result in bacteraemia and septicaemia.
- 3. Pneumothorax.** Transcutaneous aspiration of the lung causes pneumothorax in about 20% of cases; most resolve spontaneously although intercostal intubation may be required in some instances. Transient haemoptysis may also be associated with lung aspiration.
- 4. Dissemination of tumour.** Generalised dissemination of malignant cells via lymphatics and blood vessels following FNAC is a theoretical possibility but no instances have been recorded. Local dissemination by seeding of malignant cells along the needle tract is a rare complication and has been reported in cancers of the lung, prostate and pancreas. Aspiration of malignant ovarian cysts may result in release of cyst contents into the peritoneal cavity with peritoneal implants.

Precautions and Contraindications of FNAC

While FNAC is generally a safe procedure, precautions have to be taken when aspiration is contemplated of some sites under certain circumstances:

- 1. Bleeding disorders.** Thrombocytopenia *per se* is not a contraindication to FNAC. In patients with coagulopathies such as haemophilia, aspiration of joint spaces, chest and abdominal viscera is contraindicated; superficial lesions may be aspirated and pressure applied to the puncture site for at least 5 minutes following the procedure.
- 2. Liver.** Estimation of prothrombin time is an essential pre-requisite for aspiration of the liver. FNAC is not advisable if prothrombin index (PTI) is less than 80%. Obstructive jaundice is a relative contraindication for FNAC on account of the risk of bile peritonitis.

3. Lung. FNAC of the lung should not be undertaken in elderly patients with emphysema or pulmonary hypertension because of the enhanced risk of pneumothorax and haemoptysis respectively.

4. Pancreas. FNAC is contraindicated in acute pancreatitis as it aggravates the inflammatory process.

5. Prostate. Transrectal aspiration in acute prostatitis may cause bacteraemia/septicaemia and is contraindicated.

6. Testis. Aspiration is extremely painful in acute epididymo-orchitis and should be deferred till such time the acute inflammatory process subsides. The patient is treated with anti-inflammatory agents and antibiotics and FNAC undertaken at a later date.

7. Adrenal. FNAC of a suspected pheochromocytoma is inadvisable as it may sometimes provoke extreme fluctuations in blood pressure.

Limitations of FNAC

The main limitation of FNAC lies in the fact that only a small population of cells is sampled by the procedure. The reliability of the test, thus, depends upon the adequacy of the sample and its representative character. An inadequate sample which is not representative of the true lesion results in a 'false-negative' diagnosis. If the FNAC report is 'negative' despite a strong clinical suspicion of malignancy, the patient should be investigated further. FNAC may be repeated or a surgical biopsy performed to obtain a tissue diagnosis in such instances.

Lack of requisite clinical information (e.g. size, site and character of mass) or relevant investigative results (e.g. X-ray findings) further limit the utility of FNAC. Knowledge of the exact site from where the aspirate has been obtained is crucial to the accurate interpretation of FNAC smears and lack of this information severely compromises the ability of the cytopathologist to provide a diagnosis

II. IMPRINT CYTOLOGY

In imprint cytology, touch preparations from cut surfaces of fresh unfixed surgically excised mass lesions are examined. Imprints may also be obtained from draining sinuses or ulcerated areas.

For surgically resected specimens (e.g. lymph nodes) smears are prepared by bisecting or slicing the specimen and lightly touching/pressing a glass slide onto the freshly exposed surface without smearing it. Smears cannot be prepared from fixed specimens. Smears are wet-fixed or air-dried and stained as per routine.

The main advantage of the imprint smear is that the cell distribution reflects, and to some extent, recapitulates tissue architecture thus aiding in interpretation. The technique is used in the intraoperative diagnosis of malignancy as a complement to frozen-section, and is also valuable as an adjunct to histopathology in the typing of lymphomas.

III. CRUSH SMEAR CYTOLOGY

Crush smear preparations of tissue particles obtained by craniotomy have been used in the diagnosis of brain tumours. These smears are preferred by some workers as they allow recognition of tissue architecture to some degree, in addition to better cytological details.

IV. BIOPSY SEDIMENT CYTOLOGY

Biopsy sediment cytology entails the examination of sediment obtained by centrifugation of fixatives/fluids in which surgical biopsy specimens are despatched to the laboratory. The method may be useful in the rapid diagnosis of bone tumours as histological sections are usually obtained after many days on account of the delay necessitated by decalcification. For soft tissue specimens, the technique offers no particular advantage.

FIXATION AND STAINING IN CYTOLOGY

FIXATION AND FIXATIVES

All material for cytological examination must be properly fixed to ensure preservation of cytomorphological details. Methods of fixation vary depending upon the type of staining employed (e.g. Papanicolaou or Romanowsky staining). Material for exfoliative cytodiagnosis is usually *wet-fixed* (i.e. smears are immersed in fixative without allowing them to dry) for use with the Papanicolaou or haematoxylin and eosin stains. Sometimes, smears are *air-dried* for use with the Romanowsky stains (as used in haematologic studies). In Romanowsky staining, fixation is effected during the staining procedure.

1. ROUTINE FIXATIVES. The ideal fixative for routine use is Papanicolaou's fixative comprising a solution of equal parts of ether and 95% ethanol. However, the flammability of ether makes it hazardous. Most laboratories use 95% ethanol alone with excellent results. Where ethanol is not available, 100% methanol, 95% denatured alcohol, or 85% isopropyl alcohol (isopropanolol) may be used.

Smears prepared at the bedside as well as those prepared in the laboratory from fluid samples are immediately placed in 95% ethanol *without allowing them*

to dry prior to fixation. Drying causes distortion of cells and induces cytoplasmic staining artefacts. Fixation time of 10 to 15 minutes at room temperature is adequate. (Smears may also be left in the fixative for 24 hours or more without detriment to cytomorphological detail). Smears should be transported to the laboratory in the fixative (screw-capped Coplin jars are best for this purpose).

2. COATING FIXATIVES. Coating fixatives are applied as aerosol-sprays or with a dropper to the surface of freshly prepared smears. While commercial preparations (e.g. cytospray) are available, a standard hairspray with a high alcohol content is also a suitable alternative. Smears while still wet, are placed face-up on a table and sprayed with the nozzle held at a distance of 10 to 12 inches. A period of 10 minutes is sufficient for drying of coated smears, which may then be wrapped or put in a box for transport to the laboratory. Coating fixatives are ideal when unstained smears are to be transported over long distances.

3. SPECIAL PURPOSE FIXATIVES. Buffered neutral formalin, Bouin's fluid and picric acid are used for specific purposes when required. Formalin vapour fixation is also employed for some staining procedures.

4. PRESERVATION OF FLUID SAMPLES. Samples of fluids are best submitted to the laboratory in a fresh state for immediate processing. Where a delay is anticipated in despatch to the laboratory or in processing, the sample is collected in a suitable preservative for 'refixation' so that cellular morphology is preserved. When the samples have been processed and smears prepared, 'fixation' is effected as described earlier.

Fluid samples with a high protein content (e.g. pericardial, pleural and peritoneal fluids) may be preserved under refrigeration for up to 12 hours without pre-fixation. However, fluids with a low protein content (e.g. urine and cerebrospinal fluid) deteriorate in 1 to 2 hours even if refrigerated. Fluids with low pH (e.g. gastric lavage samples) must be collected in pre-cooled preservative since cells deteriorate extremely rapidly in such specimens.

The best *preservative for general use* is 50% ethanol in volumes equal to that of the sample. Ninety-five per cent ethanol precipitates proteins and hardens the sediment making smear preparation difficult; it is used only for gastric aspirates. Pericardial, pleural and peritoneal fluids may be pre-fixed with an equal volume of 10% formalin if ethanol is not available. Solutions containing ether and acetone are not used as preservatives as

they cause extreme hardening of the sediment making smear preparation virtually impossible.

STAINING OF SMEARS

Three staining procedures are commonly employed: Papanicolaou and haematoxylin and eosin (H&E) stains are used for *wet-fixed smears* while Romanowsky stains are used for *air-dried smears*.

1. PAPANICOLAOU STAIN. Papanicolaou staining is the best method for routine cytodiagnostic studies. Three solutions are used comprising a nuclear stain and two cytoplasmic counter-stains. Harris' haematoxylin is the nuclear stain. Orange G (OG-6) and eosin-alcohol (EA-65 or EA-50) are the two cytoplasmic counterstains which impart the orange and cyanophilic tints to cytoplasm respectively.

2. H&E STAIN. The stain is essentially the same as that used for histological sections. Harris' haematoxylin is the nuclear stain, and eosin is the cytoplasmic counter-stain.

3. ROMANOWSKY STAINS. Romanowsky stains used in haematological preparations may also be used for cytological smear preparations. Leishman's, May-Grünwald-Giemsa (MGG) and Wright's stains are most commonly used.

In conclusion, both exfoliative cytology and FNA cytology have now become a part of diagnostic pathology. It is imperative for the student in pathology as well as the clinician to be familiar with the advantages and limitations of cytologic diagnosis. It is acknowledged that a marked decline in incidence of cervical cancer in many western countries is attributable to highly successful preventive Pap smear screening programme. Similarly, large number of surgical diagnostic procedures are now avoided by rational use of FNAC. However, in view of unique responsibility of pathologist in patient management, reporting cytopathologist should be adequately trained in the skill and should not hesitate to ask for ancillary diagnostic techniques, or advise the use of core biopsy or open biopsy, wherever appropriate.



Function Tests

A battery of laboratory tests performed to assess the functional status of an organ is termed function test. Liver and kidneys are two such important organs where assessment of their overall function is determined by tests.

LIVER FUNCTION TESTS

In view of multiplicity and complexity of the liver functions, it is obvious that no single test can establish the disturbance in liver function. Thus a battery of liver function tests are employed for accurate diagnosis, to assess the severity of damage, to judge prognosis and to evaluate therapy. These tests are described below in relation to major liver functions.

I. TESTS FOR MANUFACTURE AND EXCRETION OF BILE

Bile is produced by the liver, stored in the gallbladder and secreted via biliary ducts into the duodenum. Bile consists of biliary phospholipids and primary and secondary bile acids. To understand the mechanisms underlying biliary pathology, it is important to understand normal bilirubin metabolism (page 356). In brief, jaundice will develop if bilirubin is excessively produced, or there is impaired hepatic uptake and conjugation of bilirubin, or it is insufficiently excreted into the duodenum. Tests employed to assess the synthesis and elimination of bilirubin pigment, urobilinogen and bile acids are outlined below:

1. BILIRUBIN. Bilirubin pigment can be detected in serum, faeces and urine.

i) Serum bilirubin estimation is based on *van den Bergh diazo reaction* by spectrophotometric method. Diazo reagent consists of diazotised sulfanilic acid. *Water-soluble conjugated* bilirubin gives *direct* van den Bergh reaction with diazo reagent within one minute, whereas *alcohol-soluble unconjugated* bilirubin is determined by *indirect* van den Bergh reaction. Addition of alcohol to the reaction mixture gives positive test for both conjugated and unconjugated bilirubin pigment. The unconjugated bilirubin level is then estimated by subtracting direct bilirubin value from this total value. The serum of normal

adults contains less than 1 mg/dl of total bilirubin, out of which less than 0.25 mg/dl is conjugated bilirubin. Bilirubin level rises in diseases of hepatocytes, obstruction to biliary excretion into duodenum, in haemolysis, and defects of hepatic uptake and conjugation of bilirubin pigment such as in Gilbert's disease.

ii) In **faeces**, excretion of bilirubin is assessed by inspection of stools. Clay-coloured stool due to absence of faecal excretion of the pigment indicates obstructive jaundice.

iii) In **urine**, conjugated bilirubin can be detected by commercially available 'dipsticks', Fouchet's test, foam test or icotest tablet method. Bilirubinuria does not occur in normal subjects nor is unconjugated bilirubin excreted in the urine. Bilirubinuria occurs only when there is raised level of conjugated bilirubin (filterable). Its excretion depends upon the level of conjugated bilirubin in plasma that is not protein-bound and is therefore available for glomerular filtration. Bilirubinuria appears in patients of hepatitis before the patient becomes jaundiced.

2. UROBILINOGEN. Urobilinogen is normally excreted in the urine. Its semiquantitative estimation in the urine can be done by preparing dilutions with Ehrlich's aldehyde reagent or by 'dipstick' method. An increase in urobilinogen in the urine is found in hepatocellular dysfunctions such as in alcoholic liver disease, cirrhosis and malignancy of the liver. It is also raised in haemolytic disease and in pyrexia. In cholestatic jaundice due to complete biliary obstruction, urobilinogen disappears from the urine.

3. BROMSULPHALEIN EXCRETION. Bromsulphalein (BSP) is a dye which is removed from circulation by the same mechanisms of binding, conjugation and excretion as bilirubin. BSP is injected intravenously and a sample of venous blood 45 minutes later is tested for percentage of injected dye remaining in the blood. The test is rarely performed nowadays because of the availability of enzyme estimations which are better indicators of hepatic dysfunction. Presently, the only value of BSP excretion test is in the diagnosis of Dubin-Johnson's syndrome (page 360).

4. BILE ACIDS (BILE SALTS). The primary bile acids (cholic acid and cheno-deoxycholic acid) are formed from cholesterol in the hepatocytes. These bile acids on secretion into the gut come in contact with colonic bacteria and undergo deconjugation with the production of secondary bile acids (deoxycholic acid and lithocholic acid). Most of these bile acids are reabsorbed through enterohepatic circulation and reach the liver. Only about 10% of the total bile acids are excreted in the faeces normally as unabsorbable toxic lithocholic acid.

Hepatobiliary diseases with cholestasis are associated with raised levels of serum bile acids which are responsible for producing itching (pruritus). These acids are excreted in the urine by active transport and passive diffusion and can be detected by simple methods as Hay's test and 'dipsticks'.

II. SERUM ENZYME ASSAYS

Determination of certain serum enzymes is considered useful in various types of liver injury, whether hepatocellular or cholestatic, as well as in quantifying liver damage. A combination of serum transaminases and alkaline phosphatase estimation is adequate to diagnose liver injury.

1. ALKALINE PHOSPHATASE. Serum alkaline phosphatase is produced by many tissues, especially bone, liver, intestine and placenta and is excreted in the bile. Most of the normal serum alkaline phosphatase (range 3-13 King-Armstrong units/dl or 25-85 IU/dl) is derived from bone. Elevation in activity of the enzyme can thus be found in diseases of bone, liver and in pregnancy. In the absence of bone disease and pregnancy, an elevated serum alkaline phosphatase levels generally reflect hepatobiliary disease. The greatest elevation (3 to 10 times normal) occurs in biliary tract obstruction. Slight to moderate increase is seen in parenchymal liver diseases such as in hepatitis and cirrhosis and in metastatic liver disease. It is possible to distinguish serum hepatic alkaline phosphatase from bony alkaline phosphatase by fractionation into isoenzymes but this is not routinely done.

2. γ -GLUTAMYL TRANSPEPTIDASE (γ -GT). The primary source of the enzyme, γ -GT, in serum is the liver. Its serum level parallels serum alkaline phosphatase and is used to confirm that the elevated serum alkaline phosphatase is of hepatobiliary origin. Besides its elevation in cholestasis and hepatocellular disease, the levels are high in patients with alcohol abuse even without liver disease.

3. TRANSAMINASES (AMINOTRANSFERASES). Assessment of liver cell necrosis is most frequently done by estimation of the following 2 serum enzymes:

i) **Serum aspartate transaminase or AST (formerly glutamic oxaloacetic transaminase or SGOT):** AST or SGOT is a *mitochondrial enzyme* released from heart, liver, skeletal muscle and kidney. Its normal serum level is 10-40 Karmen units/ml (0-35 IU).

ii) **Serum alanine transaminase or ALT (formerly glutamic pyruvic transaminase or SGPT):** ALT or SGPT is a *cytosolic enzyme* primarily present in the liver. Its normal serum level is 10-35 Karmen units/ml (0-35 IU).

Serum levels of SGOT and SGPT are increased on damage to the tissues producing them. Thus serum estimation of SGPT (ALT) which is fairly specific for liver tissue is of greater value in liver cell injury, whereas SGOT (AST) level may rise in acute necrosis or ischaemia of other organs such as the myocardium, besides liver cell injury.

Transaminase estimations are useful in the early diagnosis of viral hepatitis. Very high levels are seen in extensive acute hepatic necrosis such as in severe viral hepatitis and acute cholestasis. Alcoholic liver disease and cirrhosis are associated with mild to moderate elevation of transaminases.

4. OTHER SERUM ENZYMES. The determination of a few other serum enzymes is done sometimes but without any extra diagnostic advantage over the above mentioned enzyme assays. These are as under:

i) **5'-Nucleotidase** is another phosphatase derived from the liver. Its determination is useful to distinguish alkaline phosphatase of hepatic origin from that of bony tissue.

ii) **Lactic dehydrogenase (LDH)** is found to be elevated in serum of patients with metastatic liver involvement.

iii) **Choline esterase** synthesised by the liver is diminished in hepatocellular disease and malnutrition due to impaired synthesis.

III. TESTS FOR METABOLIC FUNCTIONS

The liver is the principal site of metabolism and synthesis of plasma proteins and amino acids, lipids and lipoproteins, carbohydrates and vitamins, besides detoxification of drugs and alcohol.

1. AMINO ACID AND PLASMA PROTEIN METABOLISM. Amino acids derived from the diet and from tissue breakdown are metabolised in the liver to ammonia and urea. A number of plasma proteins and

immunoglobulins are synthesised on polyribosomes bound to the rough endoplasmic reticulum within the hepatocytes and discharged into plasma. Based on these metabolic functions of the liver, serum estimation of proteins, immunoglobulins and ammonia and amino-aciduria are employed to assess the liver cell damage.

i) Serum proteins. Liver cells synthesise albumin, fibrinogen, prothrombin, alpha-1-antitrypsin, haptoglobin, ceruloplasmin, transferrin, alpha fetoproteins and acute phase reactant proteins. The blood levels of these plasma proteins are decreased in extensive liver damage. Routinely estimated are total concentration of serum proteins (normal 5.5 to 8 gm/dl), serum albumin (normal 3.5 to 5.5 gm/dl), serum globulin (normal 2 to 3.5 gm/dl) and albumin/globulin (A/G) ratio (normal 1.5-3:1). Electrophoresis is used to determine the proportions of α_1 , α_2 , β and γ globulins. Due to the availability of protein electrophoresis, thymol turbidity and flocculation tests based on altered plasma protein components have been discontinued.

Hypoalbuminaemia may occur in liver diseases having significant destruction of hepatocytes. Hyperglobulinaemia may be present in chronic inflammatory disorders such as in cirrhosis and chronic hepatitis.

ii) Immunoglobulins. The levels of serum immunoglobulins produced by lymphocytes and plasma cells (IgG, IgM and IgA) show nonspecific abnormalities in liver diseases and represent inflammatory or immune response rather than liver cell dysfunction. IgA is the predominant immunoglobulin in bile and its level is raised in cirrhosis, IgG is markedly raised in chronic active hepatitis and IgM is markedly increased in primary biliary cirrhosis.

iii) Clotting factors. Hepatic synthetic function of several clotting factors can be assessed by a few simple coagulation tests. Prothrombin time and partial thromboplastin time, both of which reflect the activities of various clotting factors, are prolonged in patients with hepatocellular disease. Prothrombin time is dependent upon both hepatic synthesis of clotting factors and intestinal uptake of vitamin K, a fat soluble vitamin. Thus, obstruction of the bile duct and intra-hepatic cholestasis which result in vitamin K deficiency due to impaired lipid absorption, are associated with prolonged prothrombin time. However, parenteral injection of vitamin K will normalise prothrombin time if the prolongation was due to obstruction, but there will be no improvement in prothrombin time if there is extensive hepatocellular disease.

iv) Serum ammonia. High blood levels of ammonia are found in acute fulminant hepatitis, cirrhosis and hepatic

encephalopathy. The rise in serum ammonia is due to inability of severely damaged liver to convert ammonia to urea. Thus, urea synthesis is reduced in chronic liver disease.

2. LIPID AND LIPOPROTEIN METABOLISM. Lipids synthesised in the liver include cholesterol and cholesterol esters, phospholipids and triglycerides. These lipids are insoluble in water and are carried in circulation with three major types of lipoproteins which contain apoproteins. These are: high density lipoproteins (HDL), low density lipoproteins (LDL) and very low density lipoproteins (VLDL).

Blood lipids. Estimations of total serum cholesterol, triglycerides and lipoprotein fractions are frequently done in patients with liver disease.

■ There is rise in total serum cholesterol in cholestasis, probably due to retention of cholesterol which is normally excreted in the bile (normal 130-230 mg/dl). Serum triglyceride is also elevated in cholestasis.

■ Values are lowered in acute and chronic diffuse liver diseases and in malnutrition.

3. CARBOHYDRATE METABOLISM. The liver plays a central role in carbohydrate metabolism. Blood glucose level is lowered in fulminant acute hepatic necrosis. In chronic liver disease, there is impaired glucose tolerance and relative insulin resistance.

IV. IMMUNOLOGIC TESTS

Liver diseases are associated with various immunologic abnormalities which may be nonspecific immunologic reactions or may be antibodies against specific etiologic agents.

1. NONSPECIFIC IMMUNOLOGIC REACTIONS. These include the following:

- i) *Smooth muscle antibody* to actin component of muscle is formed in certain hepatic disorders with hepatic necrosis. It appears that hepatocytes have a protein which is immunologically similar to actin.
- ii) *Mitochondrial antibody* develops in patients with primary biliary cirrhosis.
- iii) *Antinuclear antibody* is present in some patients of chronic hepatitis. The LE cell test may be positive in these cases.

2. ANTIBODIES TO SPECIFIC ETIOLOGIC AGENTS. These vary according to the etiologic agent causing the liver cell injury.

- i) *Hepatitis B surface antigen (HBsAg)* can be demonstrated in cases of serum hepatitis. A confirmed positive test for HBsAg is definite proof of hepatitis B infection.

- ii) *Hepatitis B core antibody (HBc)* can be detected in all patients with hepatitis B.
- iii) *Hepatitis B e antigen (HBeAg)* can be found in chronic varieties of hepatitis B.
- iv) *Amoeba antibodies* to *Entamoeba histolytica* develop in patients with amoebic liver abscess.

V. ANCILLARY DIAGNOSTIC TESTS

In addition to laboratory tests described above, two ancillary tests which are invariably done by the physician are ultrasonography and percutaneous liver biopsy and/or FNAC.

1. ULTRASONOGRAPHY. Ultrasound (US) examination of the liver is indicated in the following situations:

- i) Cholestasis of various etiologies to see the dilated intra- and extrahepatic canalicular tree.
- ii) Space-occupying lesions (SOLs) within the liver to determine whether they are neoplasms or non-neoplastic cysts.
- iii) To perform US-guided FNAC or liver biopsy.

2. FNAC AND/OR PERCUTANEOUS LIVER BIOPSY.

Lastly, FNAC and percutaneous liver biopsy are employed to examine the microscopic changes of hepatic morphology in various diseases. Both these tests are done after evaluation of signs of obstruction since these are contraindicated in cholestasis. FNAC and liver biopsy are otherwise easily performed bedside tests of value. Their main indications are:

- i) hepatocellular disease of unknown cause;
- ii) suspected cases of chronic hepatitis;
- iii) hepatomegaly of various etiologies;
- iv) splenomegaly of unknown cause;
- v) fever of unknown cause; and
- vi) SOLs visualised in radiologic examination.

A summary of various liver function tests is given in Table 39.1.

RENAL FUNCTION TESTS

In general, the kidney performs the following vital functions in the body:

1. *Excretion of waste products* resulting from protein metabolism.
2. *Regulation of acid-base balance* by excretion of H^+ ions (acidification) and bicarbonate ions.
3. *Regulation of salt-water balance* by hormones secreted both intra- and extra-renally.

4. *Formation of renin and erythropoietin* and thereby playing a role in the regulation of blood pressure and erythropoiesis respectively.

In order to assess renal function, a number of tests have been devised which give information regarding the following parameters:

- a) Renal blood flow
- b) Glomerular filtration
- c) Renal tubular function
- d) Urinary outflow unhindered by any obstruction.

Renal function tests are broadly divided into 4 groups (Table 39.2):

1. Urine analysis.
2. Concentration and dilution tests.
3. Blood chemistry.
4. Renal clearance tests.

In addition, renal biopsy is performed to confirm the diagnosis of renal disease. Renal biopsy is ideally fixed in alcoholic Bouin's solution and examined morphologically supported by special stains and further studies as under:

1. *Periodic acid-Schiff* stain for highlighting glomerular basement membrane.
2. *Silver impregnation* to outline the glomerular and tubular basement membrane.
3. *Immunofluorescence* to localise the antigens, complements and immunoglobulins.
4. *Electron microscopy* to see the ultrastructure of glomerular changes.

1. URINE ANALYSIS. The simplest diagnostic tests for renal function is the physical, chemical, bacteriologic and microscopic examination of the urine.

- i) The *physical examination* includes 24-hour urinary output, colour, specific gravity and osmolality.
- ii) The *chemical tests* are carried out to detect the presence of protein, glucose, red cells and haemoglobin to assess the permeability of glomerular membrane. A number of convenient dipstick tests are available for testing these chemical substances and pH. These consist of paper strips impregnated with appropriate reagents and indicator dyes.
- iii) The *bacteriologic examination* of the urine is done by proper and aseptic collection of midstream specimen of urine.
- iv) *Urine microscopy* is undertaken on a fresh unstained sample. Various components observed on microscopic examination of urine in renal disease are red cells, pus cells, epithelial cells, crystals and urinary casts.

TABLE 39.1: Liver Function Tests.

TESTS	SIGNIFICANCE
I. TESTS FOR MANUFACTURE AND EXCRETION OF BILE	
1. Bilirubin:	
i) Serum bilirubin	Increased in hepatocellular, obstructive and haemolytic disease, Gilbert's disease
ii) In faeces	Absent in biliary obstruction
iii) In urine	Conjugated bilirubinuria in patients of hepatitis
2. Urobilinogen:	Increased in hepatocellular and haemolytic diseases, absent in biliary obstruction
3. Bile acid (Bile salts):	Increased in serum and detectable in urine in cholestasis
II. SERUM ENZYME ASSAYS	
1. Alkaline phosphatase:	Increased in hepatobiliary disease (highest in biliary obstruction), bone diseases, pregnancy
2. γ-Glutamyl transpeptidase (γ-GT):	Rise parallels alkaline phosphatase but is specific for hepatobiliary diseases
3. Transaminases:	
i) SGOT (AST)	Increased in tissue injury to liver as well as to other tissues like in myocardial infarction
ii) SGPT (ALT)	Increase is fairly specific for liver cell injury
4. Other enzymes:	
i) 5'-Nucleotidase	Rise parallels alkaline phosphatase but more specific for diseases of hepatic origin
ii) Lactic dehydrogenase	Increased in tumours involving the liver
iii) Cholinesterase	Decreased in hepatocellular disease, malnutrition
III. TESTS FOR METABOLIC FUNCTIONS	
1. Amino acid and protein metabolism:	
i) Serum proteins (total, A/G ratio, protein electrophoresis)	Hypoalbuminaemia in hepatocellular diseases; hyperglobulinaemia in cirrhosis and chronic active hepatitis
ii) Immunoglobulins	Nonspecific alterations in IgA, IgG and IgM
iii) Clotting factors	Prothrombin time and partial thromboplastin time prolonged in patients with hepatocellular disease
iv) Serum ammonia	Increased in acute fulminant hepatitis, cirrhosis, hepatic encephalopathy
v) Aminoaciduria	In fulminant hepatitis
2. Lipid and lipoprotein metabolism:	
Blood lipids (total serum cholesterol, triglycerides and lipoprotein fractions)	Increased in cholestasis, decreased in acute and chronic diffuse liver disease and in malnutrition
3. Carbohydrate metabolism:	
Blood glucose and GTT	Decreased in hepatic necrosis
IV. IMMUNOLOGIC TESTS	
1. Nonspecific immunologic reactions:	
i) Smooth muscle antibody	In hepatic necrosis
ii) Mitochondrial antibody	In primary biliary cirrhosis
iii) Antinuclear antibody and LE cell test	In chronic active hepatitis
2. Antibodies to specific etiologic agents:	
i) Antibodies to hepatitis B (HBsAg, HBc, HBeAg)	In hepatitis B
ii) Amoeba antibodies	Amoebic liver abscess
V. ANCILLARY DIAGNOSTIC TESTS	
1. Ultrasound examination	Cholestasis of various etiologies; SOLs, US-guided-FNAC/liver biopsy
2. FNAC and/ or percutaneous liver biopsy	Unknown cause of hepatocellular disease, hepatomegaly and splenomegaly; long-standing hepatitis; PUO and SOLs of the liver

TABLE 39.2: Renal Function Tests.

1. **Urine analysis:**
 - i) Physical examination (output, colour, specific gravity, pH, osmolality)
 - ii) Chemical constituents (protein, glucose, red cells, haemoglobin)
 - iii) Bacteriologic examination
 - iv) Microscopy
2. **Concentration and dilution tests:**
 - i) Concentration test (fluid deprivation test)
 - ii) Dilution test (excess fluid intake test)
3. **Blood chemistry:**
 - i) Urea
 - ii) Blood urea nitrogen (BUN)
 - iii) Creatinine
4. **Renal clearance test:**
 - i) Inulin or mannitol clearance test
 - ii) Creatinine clearance
 - iii) Urea clearance
 - iv) Para-aminohippuric acid (PAH) clearance

The casts are moulded into cylindrical shapes by passage along tubules in which they are formed. They are the result of precipitation of proteins in the tubule that includes not only albumin but also the tubular secretion of the *Tamm Horsfall protein*. The latter is a high molecular weight glycoprotein normally secreted by ascending loop of Henle and DCT and probably has body defence function normally. Its secretion is increased in glomerular and tubular diseases. Casts may be *hyaline type* consisting of only proteins indicating a non-inflammatory etiology of glomerular filtration of proteins, *leucocyte casts* inflammatory in origin, or *red cell casts* from haematuria.

2. CONCENTRATION AND DILUTION TESTS.

Concentration and dilution tests are designed to evaluate functional capacity of the renal tubules. The ability of the nephron to concentrate or dilute urine is dependent upon both functional activity of the tubular cells in the renal medulla and the presence of antidiuretic hormone (ADH). Failure to achieve adequate urinary concentration can be due to either defects within the renal medulla (*nephrogenic diabetes insipidus*), or due to the lack of ADH (*central diabetes insipidus*).

Traditionally, urinary concentration is determined by specific gravity of the urine (normal range 1.003 to 1.030, average 1.018) which in cases of tubular disease remains constant at approximately 1.010 regardless of changing levels of plasma hydration. However, determination of urinary specific gravity provides only a rough estimate of osmolality of the urine. The tubular disease can be diagnosed in its early stage by *water deprivation* (concentration) or *water excess* (dilution) tests.

i) In **concentration test**, an artificial fluid deprivation is induced in the patient for more than 20 hours. If the nephron is normal, water is selectively reabsorbed resulting in excretion of urine of high solute concentration (specific gravity of 1.025 or more). However, if the tubular cells are nonfunctional, the solute concentration of the urine will remain constant regardless of stress of water deprivation.

ii) In **dilution test**, an excess of fluid is given to the patient. Normally, renal compensation should result in excretion of urine with high water content and lower solute concentration (specific gravity of 1.003 or less). If the renal tubules are diseased, the concentration of solutes in the urine will remain constant irrespective of the excess water intake.

3. BLOOD CHEMISTRY. Impairment of renal function results in elevation of end-products of protein metabolism. This includes increased accumulation of certain substances in the blood, chiefly urea (normal range 20-40 mg/dl), blood urea nitrogen (BUN) (normal range 10-20 mg/dl) and creatinine (normal range 0.5-1.5 mg/dl). An increase of these end-products in the blood is called *azotaemia*.

High levels of creatinine are associated with high levels of β_2 -microglobulin in the serum as well as urine, a low-molecular weight protein filtered excessively in the urine due to glomerular disease or due to increased production by the liver.

4. RENAL CLEARANCE TESTS. A clearance test is employed to assess the rate of glomerular filtration and the renal blood flow. The rate of this filtration can be measured by determining the excretion rate of a substance which is filtered through the glomerulus but subsequently is neither reabsorbed nor secreted by the tubules. The glomerular filtration rate (normal 120 ml/minute in an average adult) is usually equal to clearance of that substance and is calculated from the following equation:

$$C = \frac{UV}{P} \text{ where}$$

C is the clearance of the substance in ml/minute;
 U is the concentration of the substance in the urine;
 P is the concentration of the substance in the plasma; and
 V is the volume of urine passed per minute.

The substances which are used for clearance tests include inulin, mannitol, creatinine and urea.

i) In **inulin or mannitol clearance tests**, an intravenous infusion of the substance inulin or mannitol is given to maintain constant plasma concentration and accurately

timed urine samples are collected. Inulin, a mixture of fructose polymers, is considered the ideal substance for the clearance test since it is filtered from the glomerulus and is excreted unchanged in the urine.

ii) In **creatinine clearance test**, there is no need of intravenous infusion of creatinine since creatinine is normally released into plasma by muscle metabolism and a very small fraction of this substance is secreted by the tubules. The clearance of creatinine is determined by collecting urine over 24 hour period and a blood sample is withdrawn during the day. In spite of disadvantages like poor reproducibility and secretion of creatinine by the tubules, the 'endogenous' creatinine clearance test is easy and routinely employed method of estimating GFR.

iii) In **urea clearance test**, the sensitivity is much less than the creatinine or inulin clearance because plasma concentration of urea is affected by a number of factors (e.g. dietary protein, fluid intake, infection, trauma, surgery, and corticosteroids) and is partly reabsorbed by the tubules. Like in creatinine clearance, there is no need for intravenous infusion of urea.

iv) **Para-aminohippuric acid (PAH) clearance test** is employed to measure renal blood flow (unlike the preceding tests which measure GFR). PAH when infused intravenously is both filtered at the glomerulus as well as secreted by the tubules and its clearance is measured by determining its concentration in arterial blood and urine. Normally, renal blood flow is about 1200 ml per minute in an average adult.



Normal Values

Normal values in this chapter are given under two major headings:

- I. Weights and measurements of normal organs.
- II. Laboratory values of clinical significance.

I. WEIGHTS AND MEASUREMENTS OF NORMAL ORGANS

In order to understand the significance of alterations in weight and measurement of an organ in disease, it is important to be familiar with the normal values. In the

foregoing chapters normal figures are given alongside the normal structure of each organ/system that precedes the discussion of pathologic states affecting it. Here, a comprehensive list of generally accepted normal weights and measurements of most of the normal organs in fully-developed, medium-sized individual and a normal healthy newborn are compiled in Table 40.1.

Single value and value within brackets are indicative of the average figure for that organ. Measurements have been given as width × breadth (thickness) × length. An alphabetic order has been followed.

TABLE 40.1: Weights and Measurement of Normal Organs.

ORGAN	IN ADULTS	AT BIRTH (wherever applicable)
Adrenal gland:		
Weight	4–5 gm	8–11 gm
Brain:		
Weight (in males)	1400 gm	320–420 gm
Weight (in females)	1250 gm	—
Measurements (sagittal × vertical)	16.5 × 12.5 cm	—
Volume of cerebrospinal fluid	120–150 ml	—
Heart:		
Weight (in males)	300–350 gm	17–30 gm
Weight (in females)	250–300 gm	—
Thickness of right ventricular wall	0.3–0.5 cm	—
Thickness of left ventricular wall	1.3–1.5 cm	—
Circumference of mitral valve	10 cm	—
Circumference of aortic valve	7.5 cm	—
Circumference of pulmonary valve	8.5 cm	—
Circumference of tricuspid valve	12 cm	—
Volume of pericardial fluid	10–30 ml	—
Intestines:		
Length of duodenum	30 cm	—

ORGAN	IN ADULTS	AT BIRTH (wherever applicable)
Total length of small intestine	550–650 cm	—
Length of large intestine	150–170 cm	—
Kidneys:		
Weight each (in males)	150 gm	20–30 gm
Weight each (in females)	135 gm	—
Measurements	3.5 × 5.5 × 11.5 cm	—
Liver:		
Weight (in males)	1400–1600 (1500) gm	100–160 gm
Weight (in females)	1200–1400 (1300) gm	—
Measurements	27 × 8 × 20 cm	—
Lungs:		
Weight (right lung)	375–500 (450) gm	35–55 gm
Weight (left lung)	325–450 (400) gm	—
Volume of pleural fluid	< 15 ml	—
Oesophagus:		
Length (cricoid cartilage to cardia)	25 cm	—
Distance from incisors to gastro-oesophageal junction	40 cm	—
Parotid glands:		
Weight (each)	30 gm	—
Pituitary gland (hypophysis):		
Weight	500 mg	—

Contd...

TABLE 40.1: Weights and Measurement of Normal Organs (Contd)

ORGAN	IN ADULTS	AT BIRTH (wherever applicable)
Prostate:		
Weight	20 gm	—
Spleen:		
Weight	125–175 (150) gm	6–14 gm
Measurements	3.5 × 8.5 × 13 cm	
Testis and epididymis:		
Weight each (in adults)	20–27 gm	—
Thymus:		
Weight	5–10 gm	10–35 gm
Thyroid:		
Weight	15–40 gm	—

LABORATORY VALUES OF CLINICAL SIGNIFICANCE

Currently, the concept of 'normal values' and 'normal ranges' is replaced by 'reference values' and 'reference limits' in which the variables for establishing the values for the reference population in a particular test are well defined. Reference ranges are valuable guidelines for the clinician. However, the following cautions need to be exercised in their interpretation:

■ *Firstly*, they should not be regarded as absolute indicators of health and ill-health since values for

healthy individuals often overlap with values for persons afflicted with disease.

■ *Secondly*, laboratory values may vary with the method and mode of standardisation used; reference ranges given below are based on the generally accepted values by the standard methods in laboratory medicine.

■ *Thirdly*, although in most laboratories in the West and in all medical and scientific journals, international units (IU) conforming to the SI system are followed, but conventional units continue to be used in many laboratories in the developing countries.

The WHO as well as International Committee for Standardisation in Haematology (ICSH) have recommended adoption of SI system by the scientific community throughout world. In this section, laboratory values are given in both conventional and international units. Conversion from one system to the other can be done as follows:

$$\text{mg/dl} = \frac{\text{mmol/L} \times \text{atomic weight}}{10}$$

$$\text{mmol/L} = \frac{\text{mg/dl} \times 10}{\text{atomic weight}}$$

According to the SI system, the prefixes and conversion factors for metric units of length, weight and volume are as given in Table 40.2.

The laboratory values given here are divided into three sections: clinical chemistry of blood (Table 40.3), other body fluids (Table 40.4), and haematologic values (Table 40.5). In general, an alphabetic order has been followed.

TABLE 40.2: Prefixes and Conversion Factors in SI System.

PREFIX	PREFIX SYMBOL	FACTOR	UNITS OF LENGTH	UNITS OF WEIGHT	UNITS OF VOLUME
kilo-	k	10 ³	kilometre (km)	kilogram (kg)	kilolitre (kl)
		1	metre (m)	gram (g)	litre (l)
deci-	d	10 ⁻¹	decimetre (dm)	decigram (dg)	decilitre (dl)
centi-	c	10 ⁻²	centimetre (cm)	centigram (cg)	centilitre (cl)
milli-	m	10 ⁻³	millimetre (mm)	milligram (mg)	millilitre (ml)
micro-	μ	10 ⁻⁶	micrometre (μm)	microgram (μg)	microlitre (μl)
nano-	n	10 ⁻⁹	nanometre (nm)	nanogram (ng)	nanolitre (nl)
pico-	p	10 ⁻¹²	picometre (pm)	picogram (pg)	picolitre (pl)
femto-	f	10 ⁻¹⁵	femtometre (fm)	femtogram (fg)	femtolitre (fl)
atto-	a	10 ⁻¹⁸	altometre (am)	altogram (ag)	altolitre (al)

TABLE 40.3: Clinical Chemistry of Blood (Contd...)

COMPONENT	FLUID	REFERENCE VALUE	
		CONVENTIONAL	SI UNITS
Glucose (2-hr post-prandial)	Plasma		
normal		<140 mg/dl	<7.8 mmol/L
impaired glucose tolerance (IGT)		140-200 mg/dl	7.8-11.1 mmol/L
diabetes mellitus		>200 mg/dl	>11.1 mmol/L
Immunoglobulins	Serum		
IgA		90-325 mg/dl	
IgD		0-8 mg/dl	
IgE		<0.025 mg/dl	
IgG		800-1500 mg/dl	
IgM		45-150 mg/dl	
Lactate dehydrogenase (LDH)	Serum	100-190 U/L	1.7-3.2 μ kat/L
Lactate/pyruvate ratio		10/1	
Lipids	Serum	<i>See under cholesterol</i>	
Oxygen (% saturation)			
arterial blood	Whole blood	94-100%	
venous blood	Whole blood	60-85%	
pH	Blood	7.38-7.44	
Phosphatases			
acid phosphatase	Serum	0-5.5 U/L	0.90 nkat/L
alkaline phosphatase	Serum	30-120 U/L	0.5-2.0 nkat/L
Phosphorus, inorganic	Serum	3-4.5 mg/dl	1.0-1.4 mmol/L
Potassium	Serum	3.5-5.0 mEq/L	3.5-5.0 mmol/L
Proteins	Serum		
total		5.5-8 g/dl	
albumin		3.5-5.5 g/dl (50-60%)	
globulin		2.0-3.5 g/dl (40-50%)	
α 1 globulin		0.2-0.4 g/dl	
α 2 globulin		0.5-0.9 g/dl	
β globulin		0.6-1.1 g/dl	
γ globulin		0.7-1.7 g/dl	
A/G ratio		1.5-3 : 1	
Renal blood flow		1200 ml/min	
Sodium	Serum	136-145 mEq/L	136-145 mmol/L
Thyroid function tests			
radioactive iodine uptake (RAIU) 24-hr		5-30%	
thyroxine (T4)	Serum	5-12 μ g/dl	64-154 nmol/L
triiodothyronine (T3)	Serum	70-190 ng/dl	1.1-2.9 nmol/L
thyroid stimulating hormone (TSH)	Serum	0.4-5.0 μ U/ml	0.4-5.0 mU/L
Troponins, cardiac (cTn)			
troponin I (cTnI)	Serum	0-0.4 ng/ml	0-0.4 μ g/L
troponin T (cTnT)	Serum	0-0.1 ng/ml	0-0.1 μ g/L
Urea	Blood	20-40 mg/dl	3.3-6.6 mmol/L
Urea nitrogen (BUN)	Blood	10-20 mg/dl	
Uric acid	Serum		
males		2.5-8.0 mg/dl	150-480 μ mol/L
females		1.5-6.0 mg/dl	90-360 μ mol/L

TABLE 40.4: Other Body Fluids.

COMPONENT	FLUID	REFERENCE VALUE	
		CONVENTIONAL	SI UNITS
Body volume, water			
total		50-70% (60%)	
intracellular		33%	
extracellular		27%	
interstitial fluid including lymph fluid		12%	
intravascular fluid or blood plasma		5%	
fluid in mesenchymal tissues		9%	
transcellular fluid		1%	
Cerebrospinal fluid (CSF)	CSF		
CSF volume		120-150 ml	
CSF pressure		60-150 mm water	
leucocytes		0-5 lymphocytes/ μ l	
pH		7.31-7.34	
glucose		40-70 mg/dl	
proteins		20-50 mg/dl	
FIGLU	24-hr urine	<3 mg/day	<17.2 μ mol/day
Glomerular filtration rate (GFR)	Urine	180 L/day (about 125 ml/min)	
Seminal fluid	Semen		
liquefaction		Within 20 min	
sperm morphology		>70% normal, mature spermatozoa	
sperm motility		>60%	
pH		>7.0 (average 7.7)	
sperm count		60-150 million/ml	60-150 $\times 10^6$ /ml
volume		1.5-5.0 ml	
Schilling's test (intrinsic factor test)	24-hr urinary excretion	>10% of ingested dose of 'hot' vitamin B ₁₂ (<i>Details on page 460</i>)	
Urine examination	24-hr volume	600-1800 ml (variable)	
specific gravity	urine (random)	1.002-1.028 (average 1.018)	
protein excretion	24-hr urine	<150 mg/day	
protein, qualitative	urine (random)	Negative	
glucose excretion	24-hr urine	50-300 mg/day	
glucose, qualitative	urine (random)	Negative	
porphobilinogen	urine (random)	Negative	
urobilinogen	24-hr urine	1.0-3.5 mg/day	
Urobilinogen	Urine (random)	Present in 1: 20 dilution	

TABLE 40.5: Normal Haematologic Values.

COMPONENT	FLUID	REFERENCE VALUE	
		CONVENTIONAL	SI UNITS
Myelogram		<i>See Table 34.2 on page 484</i>	
Erythrocytes and Haemoglobin			
Erythrocyte count	Blood		
males		4.5-6.5 $\times 10^{12}$ /L (mean 5.5 $\times 10^{12}$ /L)	
females		3.8-5.8 $\times 10^{12}$ /L (mean 4.8 $\times 10^{12}$ /L)	
Erythrocyte diameter		6.7-7.7 μ m (mean 7.2 μ m)	

Contd...

TABLE 40.5: Normal Haematologic Values (Contd...)

COMPONENT	FLUID	REFERENCE VALUE	
		CONVENTIONAL	SI UNITS
Erythrocyte thickness			
peripheral		2.4 μm	
central		1.0 μm	
Erythrocyte indices (Absolute values)	Blood	(also see page 439)	
mean corpuscular haemoglobin (MCH)		27-32 pg	
mean corpuscular volume (MCV)		77-93 fl	
mean corpuscular haemoglobin concentration (MCHC)		30-35 g/dl	
Erythrocyte life-span	Blood	120 days	
Erythrocyte sedimentation rate (ESR)	Blood		
Westergren 1st hr, males		0-15 mm	
females		0-20 mm	
Wintrobe, 1st hr, males		0-9 mm	
females		0-20 mm	
Ferritin	Serum		
males		15-200 ng/ml	15-200 $\mu\text{g/L}$
females		12-150 ng/ml	15-150 $\mu\text{g/L}$
Haematocrit (PCV)	Blood		
males		40-54%	0.47 $\pm$ 0.07 L/L
females		37-47%	0.42 $\pm$ 0.05 L/L
Haemoglobin (Hb)			
adult haemoglobin (HbA)	Whole blood		
males		13.0-18.0 g/dl	130-180 g/L
females		11.5-16.5 g/dl	115-165 g/L
plasma Hb (quantitative)		0.5-5 mg/dl	5-50 mg/L
haemoglobin A ₂ (HbA ₂)		1.5-3.5%	
haemoglobin, foetal (HbF) in adults		<1%	
HbF, children under 6 months		<5%	
Iron, total	Serum	80-180 $\mu\text{g/dl}$	10.7-26.9 $\mu\text{mol/L}$
total iron binding capacity (TIBC)	Serum	250-460 $\mu\text{g/dl}$	44.8-71.6 $\mu\text{mol/L}$
iron saturation	Serum	20-45% (mean 33%)	
Iron intake		10-15 mg/day	
Iron loss			
males		0.5-1.0 mg/day	
females		1-2 mg/day	
Osmotic fragility	Blood		
slight haemolysis		at 0.45 to 0.39 g/dl NaCl	
complete haemolysis		at 0.33 to 0.36 g/dl NaCl	
mean corpuscular fragility		0.4-0.45 g/dl NaCl	
Reticulocytes	Blood		
adults		0.5-2.5%	
infants		2-6%	
Leucocytes in Health			
Total leucocyte count (TLC)	Blood		
adults		4,000-11,000/ μl	
infants (full term, at birth)		10,000-25,000/ μl	
infants (1 year)		6,000-16,000/ μl	

Contd...

TABLE 40.5: Normal Haematologic Values (Contd...)

COMPONENT	FLUID	REFERENCE VALUE	
		CONVENTIONAL	SI UNITS
Differential leucocyte count (DLC)	Blood film		
P (polymorphs or neutrophils)		40-75% (2,000-7,500/ μ l)	
L (lymphocytes)		20-50% (1,500-4,000/ μ l)	
M (monocytes)		2-10% (200-800/ μ l)	
E (eosinophils)		1-6% (40-400/ μ l)	
B (basophils)		< 1% (10-100/ μ l)	
Platelets and Coagulation			
Bleeding time (BT)			
Ivy's method	Prick blood	2-7 min	
template method		2.5-9.5 min	
Clot retraction time	Clotted blood		
qualitative		Visible in 60 min (complete in <24-hr)	
quantitative		48-64% (55%)	
Clotting time (CT)	Whole blood		
Lee and White method		4-9 min at 37°C	
Euglobin lysis time			72 hr
Fibrinogen	Plasma	200-400 mg/dl	2-4 g/L
Fibrin split (or degradation) products (FSP or FDP)	Plasma	<10 μ g/ml	<10 mg/L
Partial thromboplastin time with kaolin (PTTK) or activated partial thromboplastin time (APTT)	Plasma	30-40 sec	
Prothrombin time (PT) (Quick's one-stage method)	Plasma	10-14 sec	
Thrombin time (TT)	Plasma	<20 sec (control $\pm$ 2 sec)	
Platelet count	Blood	150,000-400,000/ μ l	



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